12-23-99

Box Sec 1 A

Docket No. 59896/JPW/ADM

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

ASSISTANT COMMISSIONER FOR PATENTS
Washington, D.C. 20231

December 23, 1999

S I R:

Transmitted herewith for filing are the specification and claims of the patent application of:

Kenneth A. Jones, Mary W. Walker, Joseph Tamm, Theresa A. Branchek and Christophe P.G. Gerald for Inventor(s)

CHIMERIC G PROTEINS AND USES THEREOF
Title of Invention

Also enclosed are:

X 8 sheet(s) of ____ informal X formal drawings.

X Oath or declaration of Applicant(s).

X A power of attorney

X An assignment of the invention to Synaptic Pharmaceutical Corporation

X A Preliminary Amendment

X A verified statement to establish small entity status under 37 C.F.R. §1.9 and §1.27.

The filing fee is calculated as follows:

CLAIMS AS FILED, LESS ANY CLAIMS CANCELLED BY AMENDMENT

	NUMBER FILED		NUMBER EXTRA*		RATE			FEE	
					SMALL ENTITY	OTHER ENTITY		SMALL ENTITY	OTHER ENTITY
TOTAL CLAIMS	24 -20	=	4	X	$ 9	$ 18	=	$ 36	$
Independent Claims	3 -3	=	0	X	$ 39	$ 78	=	$ 0	$
Multiple Dependent Claims Presented: ____ Yes X No					$ 130	$260	=	$ 0	$
					BASIC FEE			$ 380	$
					TOTAL FEE			$ 416	$

* If the difference in Col. 1 is less than zero, enter "0" in Col. 2

Applicants: Kenneth A. Jones et al.
Serial No.: Not Yet Known
Filed: Herewith

Letter of Transmittal
Page 2

__X__ A check in the amount of $ 416.00 to cover the filing fee.

____ Please charge Deposit Account No. _____ in the amount of $_____.

__X__ The Commissioner is hereby authorized to charge any additional fees which may be required in connection with the following or credit any over-payment to Account No. 03-3125;

__X__ Filing fees under 37 C.F.R. §1.16.

__X__ Patent application processing fees under 37 C.F.R. §1.17.

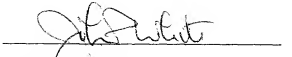
____ The issue fee set in 37 C.F.R. §1.18 at or before mailing of the Notice of Allowance, pursuant to 37 C.F.R. §1.311(b).

__X__ Three copies of this sheet are enclosed.

____ A certified copy of previously filed foreign application No. _____ filed in _____ on _____. Applicant(s) hereby claim priority based upon this aforementioned foreign application under 35 U.S.C. §119.

__X__ Other (identify) Express Mail Certificate of Mailing bearing the label No. EL066381980US, dated December 23, 1999 duplicate set of drawings, Sequence Listing, Statement in Accordance With 37 C. F. R. §1.821 (f), computer diskette containing Sequence Listing

Respectfully submitted,



John P. White
Registration No. 28,678
Attorney for Applicant(s)
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Applicant or Patentee: Kenneth A. Jones et al. Attorney's
Serial or Patent No.: Not Yet Known Docket No.: 59896/JPW/ADM
Filed or Issued: Herewith
Title of Invention or Patent: CHIMERIC G PROTEINS AND USES THEREOF

VERIFIED STATEMENT (DECLARATION) CLAIMING
SMALL ENTITY STATUS UNDER 37 C.F.R. §1.9(f)
AND §1.27(d) - SMALL BUSINESS CONCERN

I hereby declare that I am:

_____ the owner of the small business concern identified below.

__X__ an official of the small business concern empowered to act on behalf of the concern identified below:

Name of Concern: Synaptic Pharmaceutical Corporation

Address of Concern: 215 College Road
Paramus, New Jersey 07652, U.S.A.

I hereby declare that the above identified small business concern qualifies as a small business concern as defined in 13 C.F.R. §121.3-18, reproduced in 37 C.F.R. §1.9(d), for purposes of paying reduced fees under 35 U.S.C. §41(a) and §41(b), in that the number of employees of the concern, including those of its affiliates, does not exceed five hundred (500) persons. For purposes of this verified statement, the number of employees of the business concern is the average number, over the previous fiscal year, of the persons employed by the business concern on a full-time, part-time, or temporary basis during each pay period of the fiscal year, and concerns are affiliates of each other when, either directly or indirectly, one concern controls or has power to control the other, or a third party or parties controls or has power to control both.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention entitled

CHIMERIC G PROTEINS AND USES THEREOF
described in:

__X__ the specification filed herewith
_____ application serial no. _____ filed _____
_____ patent no. _____ issued _____

If the rights held by the above identified small business concern are not exclusive, each individual, concern or organization having rights to the invention is listed below[a] and no rights to the invention are held by any person, other than the inventor, who could not qualify as an independent inventor under 37 C.F.R. §1.9(c)*, any concern which could not qualify as a small business concern under 37 C.F.R. §1.9(d)* or as a nonprofit organization under 37 C.F.R. §1.9(e)*.

Name: N/A
Address: _____

____Individual ____ Small Business Concern ____ Nonprofit Organization

[a]NOTE: Separate verified statements are required for each named person, concern, or organization having rights to the invention averring to their status as small entities. 37 C.F.R. §1.27.

*See Reverse

37 C.F.R. §§1.9(c), 1.9(d), 1.9(e)

(c) An independent inventor as used in this chapter means any inventor who (1) has not assigned, granted, conveyed, or licensed, and (2) is under no obligation under contract or law to assign, grant, convey, or license, any rights in the invention to any person who could not likewise be classified as an independent inventor if that person had made the invention, or to any concern which would not qualify as a small business concern or a nonprofit organization under this section.

(d) A small business concern as used in this chapter means any business concern as defined by the Small Business Administration in 13 C.F.R. §121.3-18, published on September 30, 1982 at 47 FR 43273. For the convenience of the users of these regulations, that definition states:

§121.3-18 Definition of small business for paying reduced patent fees under Title 35, U.S. Code.

(a) Pursuant to Pub. L. 97-247, a small business concern for purposes of paying reduced fees under 35 U.S. Code 41(a) and (b) to the Patent and Trademark Office means any business concern (1) whose number of employees, including those of its affiliates, does not exceed 500 persons and (2) which has not assigned, granted, conveyed, or licensed, and is under no obligation under contract or law to assign, grant, convey or license, any rights in the invention to any person who could not be classified as an independent inventor if that person had made the invention, or to any concern which would not qualify as a small business concern or a nonprofit organization under this section. For the purpose of this section concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both. The number of employees of the business concern is the average over the fiscal year of the the persons employed during each of the pay periods of the fiscal year. Employees are those persons employed on a full-time, part-time or temporary basis during the previous fiscal year of the concern.

(b) If the Patent and Trademark Office determines that a concern is not eligible as a small business concern within this section, the concern shall have a right to appeal that determination to the Small Business Administration. The Patent and Trademark Office shall transmit its written decision and the pertinent size determination file to the SBA in the event of such adverse determination and size appeal. Such appeals by concerns should be submitted to the SBA at 1441 L Street, NW., Washington, D.C. 20416 (Attention: SBA Office of General Counsel). The appeal should state the basis upon which it is claimed that the Patent and Trademark Office initial size determination on the concern was in error; and the facts and arguments supporting the concern's claimed status as a small business concern under this section.

(e) A nonprofit organization as used in this chapter means (1) a university or other institution of higher education located in any country; (2) an organization of the type described in section 501(c)(3) of the Internal Revenue Code of 1954 (26 U.S.C. 501(c)(3)) and exempt from taxation under section 501(a) of the Internal Revenue Code (26 U.S.C. 501(a)); (3) any nonprofit scientific or educational organization qualified under a nonprofit organization statute of a state of this country (35 U.S.C. 201(i)); or (4) any nonprofit organization located in a foreign country which would qualify as a nonprofit organization under paragraphs (e)(2) or (3) of this section if it were located in this country.

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. 37 C.F.R. §1.28(b)*.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. §1001, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Name of Person Signing: Kathleen P. Mullinix
Title In Organization: President and Chief Executive Officer
Address: 215 College Road
Paramus, New Jersey 07652
Signature: Kathleen P. Mullinix
Date Of Signature: December 20, 1999

*See Reverse

37 C.F.R. §1.28(b)

(b) Once status as a small entity has been established in an application or patent, fees as a small entity may thereafter be paid in that application or patent without regard to a change in status until the issue fee is due or any maintenance fee is due. Notification of any change in status resulting in loss of entitlement to small entity status must be filed in the application or patent prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate pursuant to §1.9 of this part. The notification of change in status may be signed by the applicant, any person authorized to sign on behalf of the assignee, or an attorney or agent of record or acting in a representative capacity pursuant to §1.34(a) of this part.

Dkt. 59896/JPW/ADM

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Kenneth A. Jones, Mary W. Walker, Joseph Tamm, Theresa A. Branchek, and Christophe P.G. Gerald
U.S. Serial No.	:	Not Yet Known
Filed	:	Herewith
For	:	CHIMERIC G PROTEINS AND USES THEREOF

1185 Ave of the Americas
New York, New York 10036
December 23, 1999

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

PRELIMINARY AMENDMENT

Please amend the subject application as follows:

In the claims:

Please cancel claims 23-76, 78-140, and 142-155 without prejudice to applicants' right to pursue the subject matter of these claims in a future continuation or divisional application.

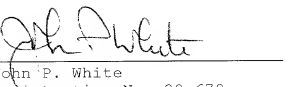
REMARKS

Claims 1-155 were pending in the subject application. By this Amendment applicants have canceled claims 23-76, 78-140, and 142-155 without prejudice or disclaimer. Accordingly, claims 1-22, 77, and 141 are currently pending.

If a telephone interview would be of assistance in advancing prosecution of the subject application, applicants' undersigned attorney invites the Examiner to telephone him at the number provided below.

No fee, other than the enclosed fee of $416.00 for filing this application, is deemed necessary in connection with the filing of this Preliminary Amendment. However, if any additional fee is required, authorization is hereby given to charge the amount of such fee to Deposit Account No. 03-3125.

Respectfully Submitted,



John P. White
Registration No. 28,678
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1185 Avenue of the Americas
New York, New York 10036
(212) 278-0400

Application
for
United States Letters Patent

To all whom it may concern:

Be it known that **Kenneth A. Jones, Mary W. Walker, Joseph Tamm, Theresa A. Branchek, and Christophe P.G. Gerald**

have invented certain new and useful improvements in

Chimeric G Proteins And Uses Thereof

of which the following is a full, clear and exact description.

CHIMERIC G PROTEINS AND USES THEREOF

BACKGROUND OF THE INVENTION

Throughout this application various publications are referred to by partial citations within parenthesis. Full citations for these publications may be found at the end of the specification immediately preceding the claims. The disclosures of these publications, in their entireties, are hereby incorporated by reference into this application in order to more fully describe the state of the art to which the invention pertains.

Therapeutic importance of G protein-coupled receptors

Intercellular communication in multicellular organisms relies on numerous signal transduction pathways that allow chemical messages to be sensed extracellularly and converted into intracellular responses. One of the most ancient and well-diversified pathways uses G protein-coupled receptors (GPCRs) as the chemical sensor. GPCRs comprise a large family of transmembrane signaling proteins that are key to a variety of cellular activities including phototransduction, olfaction, neurotransmission, and endocrine function.

There are currently about 300 molecularly identified GPCRs and this number is rapidly growing. Estimates based on genomes that have been entirely sequenced suggest that there may be more than 1000 GPCRs in humans. The fact that a large proportion of prescribed drugs act on GPCRs coupled with the evidence of a large reserve of undiscovered genes

suggests that these proteins will continue to be major targets for drug discovery for the foreseeable future.

Signaling pathways used by GPCRs

GPCRs mediate diverse cellular responses to external stimuli through their interaction with a single class of proteins known as heterotrimeric G proteins (G proteins). These proteins are composed functionally of two subunits, an α subunit that possesses GPCR-recognition and GTP-binding domains, and a dimer formed by β and γ subunits (Bourne, 1997; Lambright et al., 1996). Stimulated by agonist binding, GPCRs induce a conformational change in the G protein that facilitates the exchange of GDP for GTP bound to the α subunit. In the GTP-bound state, the α subunit is free to dissociate from the $\beta\gamma$ dimer, permitting the two subunits to independently interact with a number of membrane-bound effector proteins including enzymes and ion channels.

To date, there are 17 Gα subunits that have been cloned (Simon et al., 1991). These fall broadly into four classes: those that activate phospholipase C (Gα_q, Gα_{11}, Gα_{14}, Gα_{15}, and Gα_{16}), those that stimulate adenylate cyclase (Gα_s and isoforms), those that mediate inhibition of adenylate cyclase and also permit interaction with a variety of other effectors through release of $\beta\gamma$ subunits (Gα_i and Gα_o isoforms), and finally Gα_{12} and Gα_{13} whose regulatory functions are less well understood. By detecting and discriminating among structural features of both $\beta\gamma$ and Gα, the individual GPCR activates only a subset of available G proteins (Bourne, 1997).

The "funneling" of signaling events through specific classes of G proteins has had important consequences for the design of assays to test the functional status of a given receptor. For example, receptors that couple strongly to $G\alpha_q$, such as α_{1A}-adrenoceptors, 5-HT_{2c} receptors, or H1 histamine receptors, activate phospholipase C isoforms, initiating a rise in inositol phosphates (IP3) and a release of calcium from intracellular stores. Specific assays have been developed to measure the release of these signaling molecules. Likewise, other assays have been developed for measuring accumulation or depletion of cAMP (from stimulation or inhibition of adenylate cyclase) due to stimulation of receptors coupling either to $G\alpha_s$ or $G\alpha_i$, respectively. A myriad of other assays have been elaborated that measure ion channel, GPTγS binding, MAP kinase, or transcriptional activities. In further elaborations of these methods, artificial "reporter genes" are used to provide a simplified endpoint initiated by some of the above cellular responses.

Ligand identification for GPCR "orphan" receptors

The discovery of new GPCRs has outpaced the identification of new natural ligands, leading to a growing list of "orphan" G protein-coupled receptors whose ligand is unknown. Identifying the ligands for these orphan receptors is critical for determining their biological importance and will permit investigations into receptor pharmacology and drug design. While it is possible to identify ligands by binding, such assays depend upon the availability of high affinity radiolabeled ligands, and often on high levels of expression of the cloned receptor. On the

other hand, functional activity can be elicited using unmodified, naturally occurring ligands applied to cells expressing moderate densities of receptor. The primary disadvantage of the functional approach is not knowing which class of G protein will couple efficiently to the orphan receptor. Although much progress has been made toward identifying motifs within the intracellular portions of GPCRs that bind G proteins, currently it is not possible to predict which class of G protein will couple to a given receptor. This uncertainty requires the employment of multiple functional assays for each orphan receptor in order to cover all possible signal transduction pathways. The availability of a single, genetically modified G protein that could couple universally to the vast majority of GPCRs would be an extremely useful tool for the study of orphan receptors and for the development of new therapeutic agents targeting GPCRs.

"Promiscuous" G proteins and modified G proteins

The design of a universal functional assay for all GPCRs is a highly sought after goal for the pharmaceutical industry. Such an assay would eliminate the need to run multiple parallel assays for each receptor. Work on the $G\alpha_{16}$ subunit (Offermans and Simon, 1995) showed that a single G protein can "route" receptors that normally couple to inhibition of adenylate cyclase to stimulation of inositol phosphate production (Offermanns and Simon, 1995). Such a system can take advantage of instrumentation that detects Ca^{++} mobilization via fluorescent dyes in a multiwell plate format suitable for mass screening of compound libraries. Unfortunately, while heterologous expression systems

incorporating $G\alpha_{16}$ are amenable to mass screening, there are a significant number of GPCRs that do not couple well to this G protein, reducing its general utility for screening orphan receptors.

Studies of the three dimensional structure of native G proteins (Lambright et al., 1996) and the functional activities of chimeric G proteins (see for review, Milligan and Rees, 1999) point to two regions of the $G\alpha$ subunit that are involved in receptor recognition. Conklin and co-workers (Conklin et al., 1993) provided experimental evidence that the extreme C-terminal regions of $G\alpha_q$, $G\alpha_s$, and $G\alpha_{12}$ are important for directing targeting to the receptor. For example, replacing the last five amino acids of $G\alpha_q$ with the corresponding amino acids from $G\alpha_{12}$, permitted three receptors, which normally couple to $G\alpha_{i/o}$, to stimulate phospholipase C (PLC). Similarly, replacing with the terminal five amino acids of $G\alpha_s$, permitted stimulation of PLC by the vasopressin V2 receptor, which normally activates adenylate cyclase (Conklin et al., 1996). Other experiments, in which $G\alpha_s$ was altered by the C-terminal amino acids of $G\alpha_q$, demonstrated the generality of the finding that a given G protein can be re-directed by replacing the C-terminus of a given $G\alpha$ "backbone" with the appropriate C-terminus of another $G\alpha$ subunit (see for review, Milligan and Rees, 1999). Thus, the C-terminus of $G\alpha$ is one important determinant for GPCR recognition and may be modified to channel responses from the preferred signaling pathway to another one that would be amenable to automation.

The N-terminus of $G\alpha$ is also involved in directing G protein to a target receptor, but the specificity for

this is much less well understood. Kostenis and co-workers (Kostenis et al., 1997; Kostenis et al., 1998) noted that the N-termini of $G\alpha_q$ and $G\alpha_{11}$ are unique in that they contain a six amino acid extension not found in other $G\alpha$ subunits. Deletion of this extension permitted GPCRs that do not normally couple to wild-type $G\alpha_q$, to productively couple to the mutant and activate PLC. Although N-terminal deletion mutants of $G\alpha_q$ improve coupling to $G\alpha_{i/o}$-coupled receptors, the amplitude of second messenger response in many instances is low and not sufficient for mass screening applications.

Use of ancestral G proteins

Sequence analysis of $G\alpha$ genes from organisms spanning multiple phyla suggests the existence of a primordial $G\alpha$ ancestor (Wilkie and Yokoyama, 1994; Seack et al., 1998; Suga et al., 1999; Figure 1). Lower organisms having less elaborate second messenger pathways and effector protein targets might harbor $G\alpha$ homologues that are closer in structure to the ancestral protein. Further, these proteins may have the capacity to interact promiscuously with a wide variety of GPCRs because they lack structural motifs that subsequently evolved for the recognition of specific receptor subtypes. For example, in the search for primitive G proteins we noted that all invertebrate species, including *Caenorhabditis elegans* (*C. elegans*) and *Drosophila melanogaster* (*D. melanogaster*), lack the first six amino acids corresponding to the N-terminus of mammalian $G\alpha_q$ subunits. The use of $G\alpha$ subunits from species that appear evolutionarily early on the phylogenetic tree offers an approach to universal coupling that has not been previously described.

C. elegans is an attractive organism because its genome has been completely sequenced (The *C. elegans* Sequencing Consortium, 1998) and because, as a pseudocoelomate, it branches early in the phylogenetic tree (Keeton, 1980). *C. elegans* contains only a single homologue from each of the four major Gα families: $G\alpha_q$, $G\alpha_{i1}$, $G\alpha_s$, and $G\alpha_{12}$ (Jansen et al., 1999). This contrasts with mammals which have multiple isoforms within each of these families and, at the other phylogenetic extreme, yeast which has only two Gα subunits (Simon et al., 1991). The single $G\alpha_q$ subunit of *C. elegans* may, therefore, couple to a wider range of GPCRs than any of its mammalian homologues. When combined with specific C-terminal tails derived from mammalian non-$G\alpha_q$ subunits, the resulting chimeric G proteins may be further enhanced in their ability to efficiently couple to mammalian GPCRs.

This application describes the use of $G\alpha_q$ subunits obtained from invertebrate organisms, using *C. elegans* and *D. melanogaster* as examples, as "backbones" for the construction of chimeras. One chimera in particular, composed of *C. elegans* $G\alpha_q$ ($cG\alpha_q$) and modified to contain on its C-terminus the five amino acids of human $G\alpha_z$ ($hG\alpha_z$), exhibits surprisingly robust coupling to 78% of a large sample of cloned GPCRs. Further described are uses for this Gα chimera, and others, related to the identification of ligands for orphan GPCRs and for high-throughput screening of chemical compounds in functional assays.

SUMMARY OF THE INVENTION

The present invention provides an isolated nucleic acid encoding a chimeric G protein, wherein the chimeric G protein comprises an invertebrate Gαq G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate G protein beginning with the C-terminal amino acid of such vertebrate G protein, wherein such number equals the number of amino acids deleted or varies therefrom by no more than five amino acids, provided that at least five of the C-terminal amino acids of the chimeric G protein are present at the C-terminus of such vertebrate G protein.

The invention also provides a process for determining whether a chemical compound is a mammalian G protein-coupled receptor agonist which comprises contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor, with the compound under conditions permitting the activation of the mammalian G protein-coupled receptor, and detecting an increase in mammalian G protein-coupled receptor activity, so as to thereby determine whether the compound is a mammalian G protein-coupled receptor agonist.

The invention further provides a process for determining whether a chemical compound is a mammalian G protein-coupled receptor agonist which comprises contacting a membrane preparation from cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a

mammalian G protein-coupled receptor, with the compound under conditions permitting the activation of the mammalian G protein-coupled receptor, and detecting an increase in mammalian G protein-coupled receptor activity, so as to thereby determine whether the compound is a mammalian G protein-coupled receptor agonist.

In addition, the invention provides a process for determining whether a chemical compound is a mammalian G protein-coupled receptor antagonist which comprises contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor, with the compound in the presence of a known mammalian G protein-coupled receptor agonist, under conditions permitting the activation of the mammalian G protein-coupled receptor, and detecting a decrease in mammalian G protein-coupled receptor activity, so as to thereby determine whether the compound is a mammalian G protein-coupled receptor antagonist.

The invention further provides a process for determining whether a chemical compound is a mammalian G protein-coupled receptor antagonist which comprises contacting a membrane preparation from cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor, with the compound in the presence of a known mammalian G protein-coupled receptor agonist, under conditions permitting the activation of the mammalian G protein-coupled receptor, and detecting a decrease in mammalian G protein-coupled receptor activity, so as

to thereby determine whether the compound is a mammalian G protein-coupled receptor antagonist.

The invention also provides a process for determining whether a chemical compound specifically binds to and activates a mammalian G protein-coupled receptor, which comprises contacting cells producing a second messenger response, expressing the DNA encoding the mammalian G protein-coupled receptor, and expressing the DNA encoding a chimeric G protein, wherein such cells do not normally express the DNA encoding the chimeric G protein, with the chemical compound under conditions suitable for activation of the mammalian G protein-coupled receptor, and measuring the second messenger response in the presence and in the absence of the chemical compound, a change in the second messenger response in the presence of the chemical compound indicating that the compound activates the mammalian G protein-coupled receptor.

In addition, the invention provides a process for determining whether a chemical compound specifically binds to and inhibits activation of a mammalian G protein-coupled receptor, which comprises separately contacting cells producing a second messenger response, expressing the DNA encoding the mammalian G protein-coupled receptor, and expressing the DNA encoding a chimeric G protein, wherein such cells do not normally express the DNA encoding the chimeric G protein, with both the chemical compound and a second chemical compound known to activate the mammalian G protein-coupled receptor, and with only the second chemical compound, under conditions suitable for activation of the mammalian G protein-coupled receptor, and measuring the second messenger response in the presence of only the second chemical compound

and in the presence of both the second chemical compound and the chemical compound, a smaller change in the second messenger response in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound indicating that the chemical compound inhibits activation of the mammalian G protein-coupled receptor.

The invention further provides a process of screening a plurality of chemical compounds not known to activate a mammalian G protein-coupled receptor to identify a compound which activates the mammalian G protein-coupled receptor which comprises:

(a) contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor with the plurality of compounds not known to activate the mammalian G protein-coupled receptor, under conditions permitting activation of the mammalian G protein-coupled receptor;

(b) determining whether the activity of the mammalian G protein-coupled receptor is increased in the presence of one or more of the compounds; and if so

(c) separately determining whether the activation of the mammalian G protein-coupled receptor is increased by any compound included in the plurality of compounds, so as to thereby identify each compound which activates the mammalian G protein-coupled receptor.

The invention still further provides a process of screening a plurality of chemical compounds not known to inhibit the activation of a mammalian G protein-coupled receptor to identify a compound which inhibits the activation of the mammalian G protein-coupled receptor, which comprises:

(a) contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor with the plurality of compounds in the presence of a known mammalian G protein-coupled receptor agonist, under conditions permitting activation of the mammalian G protein-coupled receptor;

(b) determining whether the extent or amount of activation of the mammalian G protein-coupled receptor is reduced in the presence of one or more of the compounds, relative to the extent or amount of activation of the mammalian G protein-coupled receptor in the absence of such one or more compounds; and if so

(c) separately determining whether each such compound inhibits activation of the mammalian G protein-coupled receptor for each compound included in the plurality of compounds, so as to thereby identify any compound included in such plurality of compounds which inhibits the activation of the mammalian G protein-coupled receptor.

The invention also provides a process for determining whether a chemical compound is a mammalian G protein-coupled receptor agonist, which comprises separately

contacting membrane preparations from cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor with both the compound and [^{35}S]GTPγS, and with only [^{35}S]GTPγS, under conditions permitting the activation of the mammalian G protein-coupled receptor, and detecting [^{35}S]GTPγS binding to the membrane preparation and an increase in [^{35}S]GTPγS binding in the presence of the compound indicating that the chemical compound activates the mammalian G protein-coupled receptor.

In addition, the invention provides a process for determining whether a chemical compound is a mammalian G protein-coupled receptor antagonist which comprises separately contacting membrane preparations from cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor with the chemical compound, [^{35}S]GTPγS, and a second chemical compound known to activate the mammalian G protein-coupled receptor, with [^{35}S]GTPγS and only the second compound, and with [^{35}S]GTPγS alone, under conditions permitting the activation of the mammalian G protein-coupled receptor, detecting [^{35}S]GTPγS binding to each membrane preparation, comparing the increase in [^{35}S]GTPγS binding in the presence of the compound and the second compound relative to the binding of [^{35}S]GTPγS alone to the increase in [^{35}S]GTPγS binding in the presence of the second chemical compound relative to the binding of [^{35}S]GTPγS alone, and detecting a smaller increase in [^{35}S]GTPγS binding in the presence of the compound and the second compound indicating that the compound is a mammalian G protein-coupled receptor antagonist.

The invention further provides a process for determining whether a chemical compound is a mammalian G protein-coupled receptor agonist, which comprises contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor with a compound, under conditions permitting the activation of the mammalian G protein-coupled receptor, and detecting changes in receptor active state conformation as manifested by changes in receptor/G protein heterotrimer association/dissociation in the presence of the compound indicating that the chemical compound activates the mammalian G protein-coupled receptor.

The inventions still further provides a process for determining whether a chemical compound is a mammalian G protein-coupled receptor antagonist which comprises separately contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor with the chemical compound in the presence of a known mammalian G protein-coupled receptor agonist, under conditions permitting the activation of the mammalian G protein-coupled receptor, and detecting changes in receptor active state conformation as manifested by changes in receptor/G protein heterotrimer association/dissociation in the presence of the compound indicating that the compound is a mammalian G protein-coupled receptor antagonist.

The invention also provides a process for identifying a chemical compound which specifically binds to a mammalian G protein-coupled receptor which comprises

contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor, wherein such cells do not normally express the DNA encoding the chimeric G protein, with the compound under conditions suitable for binding, and detecting specific binding of the chemical compound to the mammalian G protein-coupled receptor.

The invention further provides a process for identifying a chemical compound which specifically binds to a mammalian G protein-coupled receptor which comprises contacting a membrane preparation from cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor, wherein such cells do not normally express the DNA encoding the chimeric G protein, with the compound under conditions suitable for binding, and detecting specific binding of the chemical compound to the mammalian G protein-coupled receptor.

In addition, the invention provides a process involving competitive binding for identifying a chemical compound which specifically binds to a mammalian G protein-coupled receptor which comprises separately contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor, wherein such cells do not normally express the DNA encoding the chimeric G protein, with both the chemical compound and a second chemical compound known to bind to the mammalian G protein-coupled receptor, and with only the second chemical compound, under conditions suitable for binding of both compounds, and detecting specific binding of the

chemical compound to the mammalian G protein-coupled receptor, a decrease in the binding of the second chemical compound to the mammalian G protein-coupled receptor in the presence of the chemical compound indicating that the chemical compound binds to the mammalian G protein-coupled receptor.

The invention further provides a process involving competitive binding for identifying a chemical compound which specifically binds to a mammalian G protein-coupled receptor which comprises separately contacting a membrane preparation from cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor, wherein such cells do not normally express the DNA encoding the chimeric G protein, with both the chemical compound and a second chemical compound known to bind to the receptor, and with only the second chemical compound, under conditions suitable for binding of both compounds, and detecting specific binding of the chemical compound to the mammalian G protein-coupled receptor, a decrease in the binding of the second chemical compound to the mammalian G protein-coupled receptor in the presence of the chemical compound indicating that the chemical compound binds to the mammalian G protein-coupled receptor.

The invention also provides a process of screening a plurality of chemical compounds not known to bind to a mammalian G protein-coupled receptor to identify a compound which specifically binds to the mammalian G protein-coupled receptor, which comprises

(a) contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing

DNA encoding a mammalian G protein-coupled receptor with a compound known to bind specifically to the mammalian G protein-coupled receptor;

(b) contacting the cells of step (a) with the plurality of compounds not known to bind specifically to the mammalian G protein-coupled receptor, under conditions permitting binding of compounds known to bind to the mammalian G protein-coupled receptor;

(c) determining whether the binding of the compound known to bind to the mammalian G protein-coupled receptor is reduced in the presence of the plurality of compounds, relative to the binding of the compound in the absence of the plurality of compounds; and if so

(d) separately determining the binding to the mammalian G protein-coupled receptor of each compound included in the plurality of compounds, so as to thereby identify any compound included therein which specifically binds to the mammalian G protein-coupled receptor.

The invention further provides a process of screening a plurality of chemical compounds not known to bind to a mammalian G protein-coupled receptor to identify a compound which specifically binds to the mammalian G protein-coupled receptor, which comprises

(a) contacting a membrane preparation from cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor with the

plurality of compounds not known to bind specifically to the mammalian G protein-coupled receptor under conditions permitting binding of compounds known to bind to the mammalian G protein-coupled receptor;

(b) determining whether the binding of a compound known to bind to the mammalian G protein-coupled receptor is reduced in the presence of the plurality of compounds, relative to the binding of the compound in the absence of the plurality of compounds; and if so

(c) separately determining the binding to the mammalian G protein-coupled receptor of each compound included in the plurality of compounds, so as to thereby identify any compound included therein which specifically binds to the mammalian G protein-coupled receptor.

The invention also provides a process for determining whether a chemical compound is a ligand for a mammalian G protein-coupled receptor which comprises contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor, with the compound under conditions permitting the activation of the mammalian G protein-coupled receptor, and detecting an increase in mammalian G protein-coupled receptor activity, so as to thereby determine whether the compound activates the mammalian G protein-coupled receptor and is a ligand for the mammalian G protein-coupled receptor.

The invention further provides a process for determining whether a chemical compound is a ligand

for a mammalian G protein-coupled receptor which comprises contacting a membrane preparation from cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor, with the compound under conditions permitting the activation of the mammalian G protein-coupled receptor, and detecting an increase in mammalian G protein-coupled receptor activity, so as to thereby determine whether the compound activates the mammalian G protein-coupled receptor and is a ligand for the mammalian G protein-coupled receptor.

The invention still further provides a process for determining whether a chemical compound is a ligand for a mammalian G protein-coupled receptor which comprises contacting cells producing a second messenger response, expressing the DNA encoding the mammalian G protein-coupled receptor, and expressing the DNA encoding a chimeric G protein, wherein such cells do not normally express the DNA encoding the chimeric G protein, with the chemical compound under conditions suitable for activation of the mammalian G protein-coupled receptor, and measuring the second messenger response in the presence and in the absence of the chemical compound, a change in the second messenger response in the presence of the chemical compound indicating that the compound activates the mammalian G protein-coupled receptor and is a ligand for the mammalian G protein-coupled receptor.

In addition, the invention provides a process of screening a plurality of chemical compounds not known to activate a mammalian G protein-coupled receptor to identify a ligand for the mammalian G protein-coupled receptor which comprises:

(a) contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor with the plurality of compounds not known to activate the mammalian G protein-coupled receptor, under conditions permitting activation of the mammalian G protein-coupled receptor;

(b) determining whether the activity of the mammalian G protein-coupled receptor is increased in the presence of one or more of the compounds; and if so

(c) separately determining whether the activation of the mammalian G protein-coupled receptor is increased by any compound included in the plurality of compounds, so as to thereby identify each compound which activates the mammalian G protein-coupled receptor and is a ligand for the mammalian G protein-coupled receptor.

The invention also provides a process for determining whether a chemical compound is a ligand for a mammalian G protein-coupled receptor, which comprises separately contacting membrane preparations from cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor with both the compound and [^{35}S]GTPγS, and with only [^{35}S]GTPγS, under conditions permitting the activation of the mammalian G protein-coupled receptor, and detecting [^{35}S]GTPγS binding to the membrane preparation and an increase in [^{35}S]GTPγS binding in the presence of the

compound indicating that the chemical compound activates the mammalian G protein-coupled receptor and is a ligand for the mammalian G protein-coupled receptor.

In addition, the invention provides a process for determining whether a chemical compound is a ligand for the mammalian G protein-coupled receptor, which comprises contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor with a compound, under conditions permitting the activation of the mammalian G protein-coupled receptor, and detecting changes in receptor active state conformation as manifested by changes in receptor/G protein heterotrimer association/dissociation in the presence of the compound indicating that the chemical compound activates the mammalian G protein-coupled receptor and is a ligand for the mammalian G protein-coupled receptor.

The invention further provides a process for identifying a ligand for a mammalian G protein-coupled receptor which comprises contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor, wherein such cells do not normally express the DNA encoding the chimeric G protein, with the compound under conditions suitable for binding, and detecting specific binding of the chemical compound to the mammalian G protein-coupled receptor, indicating that the compound is a ligand for the mammalian G protein-coupled receptor.

The invention still further provides a process for identifying a chemical compound which specifically binds to a mammalian G protein-coupled receptor which comprises contacting a membrane preparation from cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor, wherein such cells do not normally express the DNA encoding the chimeric G protein, with the compound under conditions suitable for binding, and detecting specific binding of the chemical compound to the mammalian G protein-coupled receptor, indicating that the compound is a ligand for the mammalian G protein-coupled receptor.

The invention also provides a process of screening a plurality of independent clones not known to include a clone encoding a mammalian G protein-coupled receptor, to identify and isolate a clone encoding a mammalian G protein-coupled receptor, which comprises:

(a) contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a plurality of independent clones with a ligand, under conditions permitting activation of a mammalian G protein-coupled receptor;

(b) determining whether the ligand activates the cells expressing the plurality of independent clones and the chimeric G protein; and if so

(c) isolating the single clone which expresses the mammalian G protein-coupled receptor activated by the ligand, so as to thereby identify any

clone included in the plurality of clones as encoding a mammalian G protein-coupled receptor.

The invention further provides a process of screening a plurality of independent clones not known to include a clone encoding a mammalian G protein-coupled receptor, to identify and isolate a clone encoding a mammalian G protein-coupled receptor, which comprises:

(a) contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a plurality of independent clones with a ligand, under conditions permitting specific binding to a mammalian G protein-coupled receptor;

(b) determining whether the ligand specifically binds to the cells expressing the plurality of independent clones and the chimeric G protein; and if so

(c) isolating the single clone which expresses the mammalian G protein-coupled receptor which specifically binds to the ligand, so as to thereby identify any clone included in the plurality of clones as encoding a mammalian G protein-coupled receptor.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1

Phylogenetic tree of the $G\alpha_q$ family of G proteins. The tree was created using the "Growtree" algorithm and specifying the "Cladogram" output (SeqLab version 10, Genetics Computer Group). Initially, a cladogram was created from a multiple sequence alignment ("Pileup" utility in SeqLab version 10, Genetics Computer Group) of all publicly available G protein sequences. The tree was then edited for clarity by removing non-$G\alpha_q$ sequences. Double tilde indicates a break in the branch to *Dictyostelium discoideum* sequences imposed to permit page formatting. Branch lengths are proportional to the number of accumulated amino acid substitutions.

GBA2_DICDI is *Dictyostelium discoideum* $G\alpha_2$ (Genbank Accession number P16051); GBA4_DICDI is *Dictyostelium discoideum* $G\alpha_4$ (Genbank Accession number P34042); GB16_MOUSE is *Mus musculus* (mouse) $G\alpha_{16}$ (Genbank Accession number G193571); GB16_HUMAN is *Homo sapiens* (human) $G\alpha_{16}$ (Genbank Accession number G182892); GBQ_PATYE is *Patinopecten yessoensis* $G\alpha_q$ (GenBank Accession number O15975); GBQ_LYMST is *Lymnaea stagnalis* $G\alpha_q$ (GenBank Accession number P38411); GBQ_HUMAN is *Homo sapiens* (human) $G\alpha_q$ (Genbank Accession number L76256); GBQ_CANFA is *Canis familiarus* $G\alpha_q$ (Genbank Accession number Q28294); GBQ_MOUSE is *Mus musculus* (mouse) $G\alpha_q$ (Genbank Accession number P21279); GBQ_XENLA is *Xenopus laevis* $G\alpha_q$ (Genbank Accession number P38410); GB11_HUMAN is *Homo sapiens* (human) $G\alpha_{11}$ (Genbank Accession number 29992); GB11_BOVIN is *Bos taurus* (bovine) $G\alpha_{11}$

(Genbank Accession number P38409); GB11_MOUSE is *Mus musculus* (mouse) $G\alpha_{11}$ (Genbank Accession number P21278); GB11_MELGA is *Meleagris gallopavo* $G\alpha_{11}$ (Genbank Accession number P45645); GB11_XENLA is *Xenopus laevis* $G\alpha_{11}$ (Genbank Accession number P43444); GBQ3_DROME is *Drosophila melanogaster* $G\alpha_{q3}$ (GenBank Accession number P54400); GBQ1_DROME is *Drosophila melanogaster* $G\alpha_{q1}$ (GenBank Accession number P23625); GBQ_HOMAM is *Homarus americanus* $G\alpha_{q}$ (GenBank Accession number P91950); GBQ_CAEEL is *Caenorhabditis elegans* $G\alpha_{q}$ (GenBank Accession number AF003739); GBQ_LOLFO is *Loligo forbesi* $G\alpha_{q}$ (GenBank Accession number P38412); GB14_MOUSE is *Mus musculus* (mouse) $G\alpha_{14}$ (Genbank Accession number P30677); GB14_BOVIN is *Bos taurus* (bovine) $G\alpha_{14}$ (Genbank Accession number P38408); and GBQ_GEOCY is *Geodia cydonium* $G\alpha_{q}$ (GenBank Accession number γ14248).

Figure 2A-2B

Amino acid sequences of $G\alpha_{q/x}$ chimeras. (*C. elegans* $G\alpha_{q/z5}$ (SEQ ID NO: 1); *C. elegans* $G\alpha_{q/z9}$ (SEQ ID NO: 2); *C. elegans* $G\alpha_{q/s9}$ (SEQ ID NO: 3); *C. elegans* $G\alpha_{q/s21}$ (SEQ ID NO: 4); *C. elegans* $G\alpha_{q/i3(5)}$ (SEQ ID NO: 5); and *D. melanogaster* $G\alpha_{q/z5}$ (SEQ ID NO: 41)). Bold regions at the C-terminus denote where amino acid substitutions are made between *C. elegans* $G\alpha_{q}$ and mammalian $G\alpha_{z}$, $G\alpha_{s}$, or $G\alpha_{i3}$. The remainder of the protein (non-bold amino acids) in each case is *C. elegans* or *D. melanogaster* $G\alpha_{q}$.

Figure 3

Examples of receptor-evoked responses in oocytes expressing $cG\alpha_{q/z5}$ or $hG\alpha_{q/z5}$ chimeric G proteins.

Figure 4

Examples of receptor-evoked responses in mammalian cells expressing $cG\alpha_{q/z5}$ or $hG\alpha_{q/z5}$ chimeric G proteins plus the human D1 receptor. Transiently transfected COS-7 cells were seeded into a 96-well microtiter plate and monitored for calcium mobilization in the FLIPR™ using the calcium-sensitive dye Fluo-3. A) Representative time course of fluorescence in cells stimulated at time = 10 seconds with 100 μM dopamine. Each curve is derived from a representative well. B) Maximal change in relative fluorescent units was calculated for dopamine concentrations ranging from 0.3 nM to 100 μM. Triplicate determinations, plotted as mean ± standard error of the mean, were used to construct concentration-response curves. In the example shown here, a measurable response to dopamine was obtained only in the presence of $cG\alpha_{q/z5}$, with a maximal signal of 14,723 fluorescence units and pEC_{50} of 6.32. Average maximal responses from multiple experiments ($n \geq 2$) are listed in Table 5.

Figures 5A-5C

Multiple sequence alignment of $G\alpha_q$ proteins from invertebrate and vertebrate organisms. Sequences were aligned using "Pileup" (SeqLab version 10, Genetics Computer Group). The degree of amino acid identity is indicated by the level of shading (black, 100% identity, white < 60%).

GBQ_HUMAN is *Homo sapiens* (human) $G\alpha_q$ (Genbank Accession number L76256; SEQ ID NO: 6); GBQ_CANFA is *Canis familiarus* $G\alpha_q$ (Genbank Accession number Q28294; SEQ ID NO: 7); GBQ_MOUSE is *Mus musculus* (mouse) $G\alpha_q$ (Genbank Accession number P21279; SEQ ID NO: 8);

GBQ_XENLA is *Xenopus laevis* $G\alpha_q$ (Genbank Accession number P38410; SEQ ID NO: 9); GBQ_PATYE is *Patinopecten yessoensis* $G\alpha_q$ (GenBank Accession number O15975; SEQ ID NO: 10); GBQ_LYMST is *Lymnaea stagnalis* $G\alpha_q$ (GenBank Accession number P38411; SEQ ID NO: 11); GBQ1_DROME is *Drosophila melanogaster* $G\alpha_{q1}$ (GenBank Accession number P23625; SEQ ID NO: 12); GBQ3_DROME is *Drosophila melanogaster* $G\alpha_{q3}$ (GenBank Accession number P54400; SEQ ID NO: 13); GBQ_HOMAM is *Homarus americanus* $G\alpha_q$ (GenBank Accession number P91950; SEQ ID NO: 14); GBQ_LIMPO is *Limulus polyphemus* $G\alpha_q$ (Genbank Accession number g1857923; SEQ ID NO: 15); GBQ_LOLFO is *Loligo forbesi* $G\alpha_q$ (GenBank Accession number P38412; SEQ ID NO: 16); GBQ_CAEEL is *Caenorhabditis elegans* $G\alpha_q$ (GenBank Accession number AF003739; SEQ ID NO: 17); GBQ_GEOCY is *Geodia cydonium* $G\alpha_q$ (GenBank Accession number 14248; SEQ ID NO: 18).

DETAILED DESCRIPTION OF THE INVENTION

The following definitions are presented as an aid in understanding this invention.

For the purposes of this invention, "ligand" is a molecule capable of binding to and modulating a receptor. The ligand may be chemically synthesized or may occur in nature.

For the purposes of this invention, "agonist" is a ligand capable of stimulating the biological activity of a receptor.

For the purposes of this invention, "antagonist" is a ligand capable of inhibiting the biological activity of a receptor.

For the purposes of this invention, "invertebrate" species are defined as those members of the Animal Kingdom that do not possess a vertebral column or backbone (Barnes, 1974).

For the purposes of this invention, in one embodiment, an invertebrate Gαq G protein has amino acids QK at positions 12 and 13 from the N-terminus and does not contain the sequence MTLESI (SEQ ID NO: 36) at the N-terminus.

For the purposes of this invention, "vertebrate" species are those members of the Animal Kingdom that do possess a vertebral column or backbone (Barnes, 1974). A common characteristic of vertebrate Gαq G proteins is an N-terminal extension composed of the amino acids MTLESI (SEQ ID NO: 36).

For the purposes of this invention, "Gαq second messenger response" is one of a number of responses which are typically produced by activation of G protein heterotrimers containing Gαq.

For the purposes of this invention, "Gαs second messenger response" is one of a number of responses which are typically produced by activation of G protein heterotrimers containing Gαs.

For the purposes of this invention, "receptor/G protein heterotrimer association/ dissociation" means a change in the intermolecular relationship between either α-β-γ subunits themselves or one or more of these subunits with the receptor.

Having due regard to the preceding definitions, the present invention provides an isolated nucleic acid encoding a chimeric G protein, wherein the chimeric G protein comprises an invertebrate Gαq G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate G protein beginning with the C-terminal amino acid of such vertebrate G protein, wherein such number equals the number of amino acids deleted or varies therefrom by no more than five amino acids, provided that at least five of the C-terminal amino acids of the chimeric G protein are present at the C-terminus of such vertebrate G protein.

In one embodiment, the nucleic acid encodes a chimeric G protein, wherein the chimeric G protein

comprises an invertebrate Gαq G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate G protein beginning with the C-terminal amino acid of such vertebrate G protein, wherein such number equals the number of amino acids deleted or varies therefrom by no more than two amino acids, provided that at least five of the C-terminal amino acids of the chimeric G protein are present at the C-terminus of such vertebrate G protein.

In another embodiment, the nucleic acid encodes a chimeric G protein, wherein the chimeric G protein comprises an invertebrate Gαq G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate G protein beginning with the C-terminal amino acid of such vertebrate G protein, wherein such number equals the number of amino acids deleted.

In one embodiment, the nucleic acid is DNA. In one embodiment, the DNA is cDNA. In another embodiment, the DNA is genomic DNA. In another embodiment, the nucleic acid is RNA.

In one embodiment, the aforementioned vertebrate G protein is a mammalian G protein. In another embodiment, the aforementioned contiguous amino acids which have been deleted are contained in FVFAAVKDTILQHNLKEYNLV* (SEQ ID NO: 37), wherein V* is the C-terminal amino acid.

In another embodiment, the vertebrate G protein is a vertebrate Gαz G protein. In another embodiment, the number of contiguous amino acids which have replaced the deleted amino acids are contained in FVFDAVTDVIIQNNLKYIGLC* (SEQ ID NO: 38), wherein C* is the C-terminal amino acid. In another embodiment, the aforementioned invertebrate Gαq G protein has five contiguous amino acids beginning with the C-terminal amino acid which have been deleted and replaced by five contiguous amino acids beginning with the C-terminal amino acid of the vertebrate Gαz protein.

In another embodiment, the vertebrate G protein is a vertebrate Gαs G protein. In another embodiment, the number of contiguous amino acids which have replaced the deleted amino acids are contained in RVFNDCRDIIQRMHLRQYELL* (SEQ ID NO: 39), wherein L* is the C-terminal amino acid. In another embodiment, the invertebrate Gαq G protein has nine contiguous amino acids beginning with the C-terminal amino acid which have been deleted and replaced by nine contiguous amino acids beginning with the C-terminal amino acid of the vertebrate Gαs protein.

In another embodiment, the vertebrate G protein is a vertebrate Gαi3 G protein. In another embodiment, the number of contiguous amino acids which have replaced the deleted amino acids are contained in FVFDAVTDVIIKNNLKECGLY* (SEQ ID NO: 40), wherein Y* is the C-terminal amino acid. In another embodiment, the invertebrate Gαq G protein has five contiguous amino acids beginning with the C-terminal amino acid which have been deleted and replaced by five

contiguous amino acids beginning with the C-terminal amino acid of the vertebrate Gαi3 protein.

In other embodiments, the vertebrate G protein is a vertebrate Gαi1 G protein, a vertebrate Gαi2 G protein, a vertebrate GαoA G protein, or a vertebrate GαoB G protein.

In another embodiment, the invertebrate Gαq G protein is a *Caenorhabditis elegans* Gαq G protein. In still other embodiments, the invertebrate Gαq G protein is a *Drosophila melanogaster* Gαq G protein, a *Limulus polyphemus* Gαq G protein, a *Patinopecten yessoensis* Gαq G protein, a *Loligo forbesi* Gαq G protein, a *Homarus americanus* Gαq G protein, a *Lymnaea stagnalis* Gαq G protein, a *Geodia cydonium* Gαq G protein, or a *Dictyostelium discoideum* $G\alpha_4$ G protein.

In other embodiments, the chimeric G protein has an amino acid sequence substantially the same as the amino acid sequence shown in (a) Figure 2, *C. elegans* $G\alpha_{q/z5}$ (SEQ ID NO: 1); (b) Figure 2, *C. elegans* $G\alpha_{q/z9}$ (SEQ ID NO: 2); (c) Figure 2, *C. elegans* $G\alpha_{q/s9}$ (SEQ ID NO: 3); (d) Figure 2, *C. elegans* $G\alpha_{q/s21}$ (SEQ ID NO: 4); (e) Figure 2, *C. elegans* $G\alpha_{q/i3(5)}$ (SEQ ID NO: 5); or (f) Figure 2, *D. melanogaster* $G\alpha_{q/z8}$ (SEQ ID NO: 41).

The invention provides a vector comprising any of the aforementioned nucleic acids. In different embodiments, the vector is adapted for expression in a cell which comprises the regulatory elements necessary for expression of the nucleic acid in the cell operatively linked to the nucleic acid encoding

the chimeric G protein so as to permit expression thereof, wherein the cell is a bacterial, amphibian, yeast, insect, or mammalian cell. In different embodiments, the vector is a plasmid, a baculovirus, or a retrovirus.

The invention provides a cell comprising any of the aforementioned vectors, wherein the cell comprises DNA encoding a mammalian G protein-coupled receptor. In one embodiment of the cell, the DNA encoding the mammalian G protein-coupled receptor is endogenous to the cell. In another embodiment, the DNA encoding the mammalian G protein-coupled receptor is transfected into the cell. In one embodiment, the cell is a non-mammalian cell. In different embodiments, the non-mammalian cell is a *Xenopus* oocyte cell or a *Xenopus* melanophore cell. In another embodiment, the cell is a mammalian cell. In different embodiments, the mammalian cell is a COS-7 cell, a 293 human embryonic kidney cell, a NIH-3T3 cell, a LM(tk-) cell, a mouse Y1 cell, or a CHO cell. In one embodiment, the cell is an insect cell. In different embodiments, the insect cell is an Sf9 cell, an Sf21 cell or a Trichoplusia *ni* 5B-4 cell. The invention provides a membrane preparation isolated from any of the aforementioned cells.

The invention also provides a process for determining whether a chemical compound is a mammalian G protein-coupled receptor agonist which comprises contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor, with the compound under conditions permitting the activation of the mammalian G protein-coupled receptor, and detecting an increase in mammalian G protein-coupled

receptor activity, so as to thereby determine whether the compound is a mammalian G protein-coupled receptor agonist.

The invention further provides a process for determining whether a chemical compound is a mammalian G protein-coupled receptor agonist which comprises contacting a membrane preparation from cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor, with the compound under conditions permitting the activation of the mammalian G protein-coupled receptor, and detecting an increase in mammalian G protein-coupled receptor activity, so as to thereby determine whether the compound is a mammalian G protein-coupled receptor agonist.

The invention also provides a process for determining whether a chemical compound is a mammalian G protein-coupled receptor antagonist which comprises contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor, with the compound in the presence of a known mammalian G protein-coupled receptor agonist, under conditions permitting the activation of the mammalian G protein-coupled receptor, and detecting a decrease in mammalian G protein-coupled receptor activity, so as to thereby determine whether the compound is a mammalian G protein-coupled receptor antagonist.

The invention further provides a process for determining whether a chemical compound is a mammalian G protein-coupled receptor antagonist which comprises contacting a membrane preparation from

cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor, with the compound in the presence of a known mammalian G protein-coupled receptor agonist, under conditions permitting the activation of the mammalian G protein-coupled receptor, and detecting a decrease in mammalian G protein-coupled receptor activity, so as to thereby determine whether the compound is a mammalian G protein-coupled receptor antagonist.

In one embodiment of any of the aforementioned processes, the DNA encoding the mammalian G protein-coupled receptor is endogenous to the cell. In another embodiment, the DNA encoding the mammalian G protein-coupled receptor is transfected into the cell. In different embodiments, the mammalian G protein-coupled receptor is a human Y5 receptor, a human GALR2 receptor, a human kappa opioid receptor, a human NPFF1 receptor, a human NPFF2 receptor, a human α2A adrenergic receptor, a human dopamine D2 receptor, a human GALR1 receptor, a human Y2 receptor, a human Y1 receptor, a human Y4 receptor, a human α1A adrenergic receptor, a human dopamine D1 receptor, or a rat NTR1 receptor.

The invention also provides a process for determining whether a chemical compound specifically binds to and activates a mammalian G protein-coupled receptor, which comprises contacting cells producing a second messenger response, expressing the DNA encoding the mammalian G protein-coupled receptor, and expressing the DNA encoding a chimeric G protein, wherein such cells do not normally express the DNA encoding the chimeric G protein, with the chemical compound under conditions suitable for activation of the mammalian G

protein-coupled receptor, and measuring the second messenger response in the presence and in the absence of the chemical compound, a change in the second messenger response in the presence of the chemical compound indicating that the compound activates the mammalian G protein-coupled receptor. In one embodiment of the process, the DNA encoding the mammalian G protein-coupled receptor is endogenous to the cell. In another embodiment, DNA encoding the mammalian G protein-coupled receptor is transfected into the cell.

In one embodiment of the aforementioned process, the second messenger response is the detection of a reporter protein under the transcriptional control of a promoter element. In another embodiment, the second messenger response is measured by a change in cell proliferation. In another embodiment, the second messenger response is a Gαq second messenger response. In one embodiment, the Gαq second messenger response comprises release of inositol phosphate and the change in second messenger is an increase in the level of inositol phosphate. In another embodiment, the Gαq second messenger response comprises release of arachidonic acid and the change in second messenger is an increase in the level of arachidonic acid. In another embodiment, the Gαq second messenger response comprises activation of MAP kinase and the change in second messenger response is an increase in MAP kinase activation. In another embodiment, the Gαq second messenger response comprises intracellular calcium levels and the change in second messenger is an increase in the measure of intracellular calcium. In one embodiment, the measure of intracellular calcium levels is made by

chloride current readings. In other embodiments, the measure of intracellular calcium is made by fluorescence readings, luminescence readings, electrophysiological readings, or through the detection of a reporter protein under the transcriptional control of a calcium-responsive promoter element.

In addition, the invention provides a process for determining whether a chemical compound specifically binds to and inhibits activation of a mammalian G protein-coupled receptor, which comprises separately contacting cells producing a second messenger response, expressing the DNA encoding the mammalian G protein-coupled receptor, and expressing the DNA encoding a chimeric G protein, wherein such cells do not normally express the DNA encoding the chimeric G protein, with both the chemical compound and a second chemical compound known to activate the mammalian G protein-coupled receptor, and with only the second chemical compound, under conditions suitable for activation of the mammalian G protein-coupled receptor, and measuring the second messenger response in the presence of only the second chemical compound and in the presence of both the second chemical compound and the chemical compound, a smaller change in the second messenger response in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound indicating that the chemical compound inhibits activation of the mammalian G protein-coupled receptor. In one embodiment of the process, the DNA encoding the mammalian G protein-coupled receptor is endogenous to the cell. In another embodiment, DNA encoding the mammalian G

protein-coupled receptor is transfected into the cell.

In one embodiment of the aforementioned process, the second messenger response is the detection of a reporter protein under the transcriptional control of a promoter element. In another embodiment, the second messenger response is measured by a change in cell proliferation. In another embodiment, the second messenger response is a Gαq second messenger response. In one embodiment, the Gαq second messenger response comprises release of inositol phosphate and the change in second messenger response is a smaller increase in the level of inositol phosphate in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound. In another embodiment, the Gαq second messenger response comprises activation of MAP kinase and the change in second messenger response is a smaller increase in the level of MAP kinase activation in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound. In another embodiment, the Gαq second messenger response comprises release of arachidonic acid and the change in second messenger response is an increase in the level of arachidonic acid levels in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound. In another embodiment, the Gαq second messenger response comprises change in intracellular calcium levels and the change in second messenger response is a smaller increase in the measure of intracellular calcium in the presence of both the chemical compound and the

second chemical compound than in the presence of only the second chemical compound. In one embodiment, the measure of intracellular calcium levels is made by chloride current readings. In other embodiments, the measure of intracellular calcium is made by fluorescence readings, luminescence readings, electrophysiological readings, or through the detection of a reporter protein under the transcriptional control of a calcium-responsive promoter element.

The invention also provides a process of screening a plurality of chemical compounds not known to activate a mammalian G protein-coupled receptor to identify a compound which activates the mammalian G protein-coupled receptor which comprises:

(a) contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor with the plurality of compounds not known to activate the mammalian G protein-coupled receptor, under conditions permitting activation of the mammalian G protein-coupled receptor;

(b) determining whether the activity of the mammalian G protein-coupled receptor is increased in the presence of one or more of the compounds; and if so

(c) separately determining whether the activation of the mammalian G protein-coupled receptor is increased by any compound included in the plurality of compounds, so as to thereby

identify each compound which activates the mammalian G protein-coupled receptor.

The invention further provides a process of screening a plurality of chemical compounds not known to inhibit the activation of a mammalian G protein-coupled receptor to identify a compound which inhibits the activation of the mammalian G protein-coupled receptor, which comprises:

(a) contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor with the plurality of compounds in the presence of a known mammalian G protein-coupled receptor agonist, under conditions permitting activation of the mammalian G protein-coupled receptor;

(b) determining whether the extent or amount of activation of the mammalian G protein-coupled receptor is reduced in the presence of one or more of the compounds, relative to the extent or amount of activation of the mammalian G protein-coupled receptor in the absence of such one or more compounds; and if so

(c) separately determining whether each such compound inhibits activation of the mammalian G protein-coupled receptor for each compound included in the plurality of compounds, so as to thereby identify any compound included in such plurality of compounds which inhibits the activation of the mammalian G protein-coupled receptor.

In one embodiment of the aforementioned processes, the DNA encoding the mammalian G protein-coupled receptor is endogenous to the cell. In another embodiment, the DNA encoding the mammalian G protein-coupled receptor is transfected into the cell.

The invention also provides a process for determining whether a chemical compound is a mammalian G protein-coupled receptor agonist, which comprises separately contacting membrane preparations from cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor with both the compound and [^{35}S]GTPγS, and with only [^{35}S]GTPγS, under conditions permitting the activation of the mammalian G protein-coupled receptor, and detecting [^{35}S]GTPγS binding to the membrane preparation and an increase in [^{35}S]GTPγS binding in the presence of the compound indicating that the chemical compound activates the mammalian G protein-coupled receptor.

The invention further provides a process for determining whether a chemical compound is a mammalian G protein-coupled receptor antagonist which comprises separately contacting membrane preparations from cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor with the chemical compound, [^{35}S]GTPγS, and a second chemical compound known to activate the mammalian G protein-coupled receptor, with [^{35}S]GTPγS and only the second compound, and with [^{35}S]GTPγS alone, under conditions permitting the activation of the mammalian G protein-coupled receptor, detecting [^{35}S]GTPγS binding to each membrane preparation, comparing the

increase in [^{35}S]GTPγS binding in the presence of the compound and the second compound relative to the binding of [^{35}S]GTPγS alone to the increase in [^{35}S]GTPγS binding in the presence of the second chemical compound relative to the binding of [^{35}S]GTPγS alone, and detecting a smaller increase in [^{35}S]GTPγS binding in the presence of the compound and the second compound indicating that the compound is a mammalian G protein-coupled receptor antagonist.

In one embodiment of the aforementioned processes, the DNA encoding the mammalian G protein-coupled receptor is endogenous to the cell. In another embodiment, the DNA encoding the mammalian G protein-coupled receptor is transfected into the cell. In one embodiment, the mammalian G protein-coupled receptor produces a Gαs second messenger response in the absence of the chimeric G protein.

This invention also provides a process for determining whether a chemical compound is a mammalian G protein-coupled receptor agonist, which comprises contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor with a compound, under conditions permitting the activation of the mammalian G protein-coupled receptor, and detecting changes in receptor active state conformation as manifested by changes in receptor/G protein heterotrimer association/dissociation in the presence of the compound indicating that the chemical compound activates the mammalian G protein-coupled receptor.

This invention further provides a process for determining whether a chemical compound is a mammalian G protein-coupled receptor antagonist which comprises separately contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor with the chemical compound in the presence of a known mammalian G protein-coupled receptor agonist, under conditions permitting the activation of the mammalian G protein-coupled receptor, and detecting changes in receptor active state conformation as manifested by changes in receptor/G protein heterotrimer association/dissociation in the presence of the compound indicating that the compound is a mammalian G protein-coupled receptor antagonist.

In one embodiment of the aforementioned processes, the DNA encoding the mammalian G protein-coupled receptor is endogenous to the cell. In another embodiment, the DNA encoding the mammalian G protein-coupled receptor is transfected into the cell.

In one embodiment of any of the aforementioned processes, the chimeric G protein comprises an invertebrate Gαq G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate G protein beginning with the C-terminal amino acid of such vertebrate G protein, wherein such number equals the number of amino acids deleted. In another embodiment, the chimeric G protein comprises an invertebrate Gαq G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the

C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate Gαz protein beginning with the C-terminal amino acid of such vertebrate Gαz protein, wherein such number equals the number of amino acids deleted. In another embodiment, the chimeric G protein comprises an invertebrate Gαq G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate Gαs protein beginning with the C-terminal amino acid of such vertebrate Gαs protein, wherein such number equals the number of amino acids deleted. In another embodiment, the chimeric G protein comprises an invertebrate Gαq G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate Gαi3 protein beginning with the C-terminal amino acid of such vertebrate Gαi3 protein, wherein such number equals the number of amino acids deleted.

In another embodiment, the chimeric G protein comprises a *Caenorhabditis elegans* Gαq G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate G protein beginning with the C-terminal amino acid of such vertebrate G protein, wherein such number equals the number of amino acids deleted. In other embodiments, the chimeric G protein comprises a *Drosophila melanogaster* Gαq G protein, a *Limulus*

polyphemus Gαq G protein, a *Patinopecten yessoensis* Gαq G protein, a *Loligo forbesi* Gαq G protein, a *Homarus americanus* Gαq G protein, a *Lymnaea stagnalis* Gαq G protein, a *Geodia cydonium* Gαq G protein, or a *Dictyostelium discoideum* $G\alpha_4$ G protein, from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate G protein beginning with the C-terminal amino acid of a vertebrate G protein, wherein such number equals the number of amino acids deleted. In other embodiments, the chimeric G protein has an amino acid sequence substantially the same as the amino acid sequence shown in (a) Figure 2, *C. elegans* $G\alpha_{q/z5}$ (SEQ ID NO: 1); (b) Figure 2, *C. elegans* $G\alpha_{q/z9}$ (SEQ ID NO: 2); (c) Figure 2, *C. elegans* $G\alpha_{q/s9}$ (SEQ ID NO: 3); (d) Figure 2, *C. elegans* $G\alpha_{q/s21}$ (SEQ ID NO: 4); (e) Figure 2, *C. elegans* $G\alpha_{q/i3(5)}$ (SEQ ID NO: 5); or (f) Figure 2, *D. melanogaster* $G\alpha_{q/z5}$ (SEQ ID NO: 41).

In one embodiment of any of the aforementioned processes, the cell is an insect cell. In another embodiment, the cell is a mammalian cell. In one embodiment, the mammalian cell is nonneuronal in origin. In further embodiments, the nonneuronal cell is a COS-7 cell, 293 human embryonic kidney cell, a CHO cell, a NIH-3T3 cell, a mouse Y1 cell, or a LM(tk-) cell.

The invention also provides a process for identifying a chemical compound which specifically binds to a mammalian G protein-coupled receptor which comprises contacting cells transfected with and expressing DNA

encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor, wherein such cells do not normally express the DNA encoding the chimeric G protein, with the compound under conditions suitable for binding, and detecting specific binding of the chemical compound to the mammalian G protein-coupled receptor.

The invention further provides a process for identifying a chemical compound which specifically binds to a mammalian G protein-coupled receptor which comprises contacting a membrane preparation from cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor, wherein such cells do not normally express the DNA encoding the chimeric G protein, with the compound under conditions suitable for binding, and detecting specific binding of the chemical compound to the mammalian G protein-coupled receptor.

In addition, the invention provides a process involving competitive binding for identifying a chemical compound which specifically binds to a mammalian G protein-coupled receptor which comprises separately contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor, wherein such cells do not normally express the DNA encoding the chimeric G protein, with both the chemical compound and a second chemical compound known to bind to the mammalian G protein-coupled receptor, and with only the second chemical compound, under conditions suitable for binding of both compounds, and detecting specific binding of the chemical compound to the mammalian G protein-coupled

receptor, a decrease in the binding of the second chemical compound to the mammalian G protein-coupled receptor in the presence of the chemical compound indicating that the chemical compound binds to the mammalian G protein-coupled receptor.

The invention further provides a process involving competitive binding for identifying a chemical compound which specifically binds to a mammalian G protein-coupled receptor which comprises separately contacting a membrane preparation from cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor, wherein such cells do not normally express the DNA encoding the chimeric G protein, with both the chemical compound and a second chemical compound known to bind to the receptor, and with only the second chemical compound, under conditions suitable for binding of both compounds, and detecting specific binding of the chemical compound to the mammalian G protein-coupled receptor, a decrease in the binding of the second chemical compound to the mammalian G protein-coupled receptor in the presence of the chemical compound indicating that the chemical compound binds to the mammalian G protein-coupled receptor.

The invention also provides a process of screening a plurality of chemical compounds not known to bind to a mammalian G protein-coupled receptor to identify a compound which specifically binds to the mammalian G protein-coupled receptor, which comprises

(a) contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled

receptor with a compound known to bind specifically to the mammalian G protein-coupled receptor;

(b) contacting the cells of step (a) with the plurality of compounds not known to bind specifically to the mammalian G protein-coupled receptor, under conditions permitting binding of compounds known to bind to the mammalian G protein-coupled receptor;

(c) determining whether the binding of the compound known to bind to the mammalian G protein-coupled receptor is reduced in the presence of the plurality of compounds, relative to the binding of the compound in the absence of the plurality of compounds; and if so

(d) separately determining the binding to the mammalian G protein-coupled receptor of each compound included in the plurality of compounds, so as to thereby identify any compound included therein which specifically binds to the mammalian G protein-coupled receptor.

The invention further provides a process of screening a plurality of chemical compounds not known to bind to a mammalian G protein-coupled receptor to identify a compound which specifically binds to the mammalian G protein-coupled receptor, which comprises

(a) contacting a membrane preparation from cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor with the plurality of compounds not known to bind

specifically to the mammalian G protein-coupled receptor under conditions permitting binding of compounds known to bind to the mammalian G protein-coupled receptor;

(b) determining whether the binding of a compound known to bind to the mammalian G protein-coupled receptor is reduced in the presence of the plurality of compounds, relative to the binding of the compound in the absence of the plurality of compounds; and if so

(c) separately determining the binding to the mammalian G protein-coupled receptor of each compound included in the plurality of compounds, so as to thereby identify any compound included therein which specifically binds to the mammalian G protein-coupled receptor.

In one embodiment of any of the aforementioned processes, the DNA encoding the mammalian G protein-coupled receptor is endogenous to the cell. In another embodiment, the DNA encoding the mammalian G protein-coupled receptor is transfected into the cell.

In one embodiment of any of the aforementioned processes, the chimeric G protein comprises an invertebrate Gαq G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate G protein beginning with the C-terminal amino acid of such vertebrate G protein, wherein such number equals the number of amino acids deleted. In another embodiment, the

chimeric G protein comprises an invertebrate Gαq G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate Gαz protein beginning with the C-terminal amino acid of such vertebrate Gαz protein, wherein such number equals the number of amino acids deleted. In another embodiment, the chimeric G protein comprises an invertebrate Gαq G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate Gαs protein beginning with the C-terminal amino acid of such vertebrate Gαs protein, wherein such number equals the number of amino acids deleted. In another embodiment, the chimeric G protein comprises an invertebrate Gαq G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate Gαi3 protein beginning with the C-terminal amino acid of such vertebrate Gαi3 protein, wherein such number equals the number of amino acids deleted.

In another embodiment, the chimeric G protein comprises an *Caenorhabditis elegans* Gαq G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate G protein beginning with the C-terminal amino acid of such vertebrate G protein, wherein such

number equals the number of amino acids deleted. In other embodiments, the chimeric G protein comprises a *Drosophila melanogaster* Gαq G protein, a *Limulus polyphemus* Gαq G protein, a *Patinopecten yessoensis* Gαq G protein, a *Loligo forbesi* Gαq G protein, a *Homarus americanus* Gαq G protein, a *Lymnaea stagnalis* Gαq G protein, a *Geodia cydonium* Gαq G protein, or a *Dictyostelium discoideum* $G\alpha_4$ G protein, from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate G protein beginning with the C-terminal amino acid of such vertebrate G protein, wherein such number equals the number of amino acids deleted. In other embodiments, the chimeric G protein has an amino acid sequence substantially the same as the amino acid sequence shown in (a) Figure 2, *C. elegans* $G\alpha_{q/z5}$ (SEQ ID NO: 1); (b) Figure 2, *C. elegans* $G\alpha_{q/z9}$ (SEQ ID NO: 2); (c) Figure 2, *C. elegans* $G\alpha_{q/s9}$ (SEQ ID NO: 3); (d) Figure 2, *C. elegans* $G\alpha_{q/s21}$ (SEQ ID NO: 4); (e) Figure 2, *C. elegans* $G\alpha_{q/i3(5)}$ (SEQ ID NO: 5); or (f) Figure 2, *D. melanogaster* $G\alpha_{q/z5}$ (SEQ ID NO: 41).

In one embodiment of any of the aforementioned processes, the cell is an insect cell. In another embodiment, the cell is a mammalian cell. In one embodiment, the mammalian cell is nonneuronal in origin. In further embodiments, the nonneuronal cell is a COS-7 cell, 293 human embryonic kidney cell, a CHO cell, a NIH-3T3 cell, a mouse Y1 cell, or a LM(tk-) cell.

The invention provides a process for determining whether a chemical compound is a ligand for a mammalian G protein-coupled receptor which comprises contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor, with the compound under conditions permitting the activation of the mammalian G protein-coupled receptor, and detecting an increase in mammalian G protein-coupled receptor activity, so as to thereby determine whether the compound activates the mammalian G protein-coupled receptor and is a ligand for the mammalian G protein-coupled receptor.

The invention further provides a process for determining whether a chemical compound is a ligand for a mammalian G protein-coupled receptor which comprises contacting a membrane preparation from cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor, with the compound under conditions permitting the activation of the mammalian G protein-coupled receptor, and detecting an increase in mammalian G protein-coupled receptor activity, so as to thereby determine whether the compound activates the mammalian G protein-coupled receptor and is a ligand for the mammalian G protein-coupled receptor.

The invention also provides a process for determining whether a chemical compound is a ligand for a mammalian G protein-coupled receptor which comprises contacting cells producing a second messenger response, expressing the DNA encoding the mammalian G protein-coupled receptor, and expressing the DNA encoding a chimeric G protein, wherein such cells do

not normally express the DNA encoding the chimeric G protein, with the chemical compound under conditions suitable for activation of the mammalian G protein-coupled receptor, and measuring the second messenger response in the presence and in the absence of the chemical compound, a change in the second messenger response in the presence of the chemical compound indicating that the compound activates the mammalian G protein-coupled receptor and is a ligand for the mammalian G protein-coupled receptor.

In one embodiment of the aforementioned process, the second messenger response is a Gαq second messenger response. In one embodiment, the Gαq second messenger response comprises intracellular calcium levels and the change in second messenger is an increase in the measure of intracellular calcium. In one embodiment, the measure of intracellular calcium levels is made by chloride current readings. In other embodiments, the measure of intracellular calcium is made by fluorescence readings, luminescence readings, electrophysiological readings, or through the detection of a reporter protein under the transcriptional control of a calcium-responsive promoter element.

In addition, the invention provides a process of screening a plurality of chemical compounds not known to activate a mammalian G protein-coupled receptor to identify a ligand for the mammalian G protein-coupled receptor which comprises:

(a) contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor with the plurality of compounds not

known to activate the mammalian G protein-coupled receptor, under conditions permitting activation of the mammalian G protein-coupled receptor;

(b) determining whether the activity of the mammalian G protein-coupled receptor is increased in the presence of one or more of the compounds; and if so

(c) separately determining whether the activation of the mammalian G protein-coupled receptor is increased by any compound included in the plurality of compounds, so as to thereby identify each compound which activates the mammalian G protein-coupled receptor and is a ligand for the mammalian G protein-coupled receptor.

The invention also provides a process for determining whether a chemical compound is a ligand for a mammalian G protein-coupled receptor, which comprises separately contacting membrane preparations from cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor with both the compound and [^{35}S]GTPγS, and with only [^{35}S]GTPγS, under conditions permitting the activation of the mammalian G protein-coupled receptor, and detecting [^{35}S]GTPγS binding to the membrane preparation and an increase in [^{35}S]GTPγS binding in the presence of the compound indicating that the chemical compound activates the mammalian G protein-coupled receptor and is a ligand for the mammalian G protein-coupled receptor.

In addition, the invention provides a process for determining whether a chemical compound is a ligand for the mammalian G protein-coupled receptor, which comprises contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor with a compound, under conditions permitting the activation of the mammalian G protein-coupled receptor, and detecting changes in receptor active state conformation as manifested by changes in receptor/G protein heterotrimer association/dissociation in the presence of the compound indicating that the chemical compound activates the mammalian G protein-coupled receptor and is a ligand for the mammalian G protein-coupled receptor.

The invention further provides a process for identifying a ligand for a mammalian G protein-coupled receptor which comprises contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor, wherein such cells do not normally express the DNA encoding the chimeric G protein, with the compound under conditions suitable for binding, and detecting specific binding of the chemical compound to the mammalian G protein-coupled receptor, indicating that the compound is a ligand for the mammalian G protein-coupled receptor.

The inventions still further provides a process for identifying a chemical compound which specifically binds to a mammalian G protein-coupled receptor which comprises contacting a membrane preparation from cells transfected with and expressing DNA encoding a

chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor, wherein such cells do not normally express the DNA encoding the chimeric G protein, with the compound under conditions suitable for binding, and detecting specific binding of the chemical compound to the mammalian G protein-coupled receptor, indicating that the compound is a ligand for the mammalian G protein-coupled receptor.

In one embodiment of any of the aforementioned processes, the DNA encoding the mammalian G protein-coupled receptor is endogenous to the cell. In another embodiment, the DNA encoding the mammalian G protein-coupled receptor is transfected into the cell.

In one embodiment of any of the aforementioned processes, the chimeric G protein comprises an invertebrate Gαq G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate G protein beginning with the C-terminal amino acid of such vertebrate G protein, wherein such number equals the number of amino acids deleted. In another embodiment, the chimeric G protein comprises an invertebrate Gαq G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate Gαz protein beginning with the C-terminal amino acid of such vertebrate Gαz protein, wherein such number equals the number of amino acids deleted.

In another embodiment, the chimeric G protein comprises an invertebrate Gαq G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate Gαs protein beginning with the C-terminal amino acid of such vertebrate Gαs protein, wherein such number equals the number of amino acids deleted. In another embodiment, the chimeric G protein comprises an invertebrate Gαq G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate Gi3 protein beginning with the C-terminal amino acid of such vertebrate Gi3 protein, wherein such number equals the number of amino acids deleted.

In another embodiment, the chimeric G protein comprises an *Caenorhabditis elegans* Gαq G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate G protein beginning with the C-terminal amino acid of such vertebrate G protein, wherein such number equals the number of amino acids deleted. In other embodiments, the chimeric G protein comprises a *Drosophila melanogaster* Gαq G protein, a *Limulus polyphemus* Gαq G protein, a *Patinopecten yessoensis* Gαq G protein, a *Loligo forbesi* Gαq G protein, a *Homarus americanus* Gαq G protein, a *Lymnaea stagnalis* Gαq G protein, a *Geodia cydonium* Gαq G protein, or a *Dictyostelium discoideum* $G\alpha_4$ G protein, from which at

least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate G protein beginning with the C-terminal amino acid of such vertebrate G protein, wherein such number equals the number of amino acids deleted. In other embodiments, the chimeric G protein has an amino acid sequence substantially the same as the amino acid sequence shown in (a) Figure 2, *C. elegans* $G\alpha_{q/z5}$ (SEQ ID NO: 1); (b) Figure 2, *C. elegans* $G\alpha_{q/z9}$ (SEQ ID NO: 2); (c) Figure 2, *C. elegans* $G\alpha_{q/s9}$ (SEQ ID NO: 3); (d) Figure 2, *C. elegans* $G\alpha_{q/s21}$ (SEQ ID NO: 4); (e) Figure 2, *C. elegans* $G\alpha_{q/i3(5)}$ (SEQ ID NO: 5); or (f) Figure 2, *D. melanogaster* $G\alpha_{q/zs}$ (SEQ ID NO: 41).

In one embodiment of any of the aforementioned processes, the cell is an insect cell. In another embodiment, the cell is a mammalian cell. In one embodiment, the mammalian cell is nonneuronal in origin. In further embodiments, the nonneuronal cell is a COS-7 cell, 293 human embryonic kidney cell, a CHO cell, a NIH-3T3 cell, a mouse Y1 cell, or a LM(tk-) cell.

The invention also provides a process of screening a plurality of independent clones not known to include a clone encoding a mammalian G protein-coupled receptor, to identify and isolate a clone encoding a mammalian G protein-coupled receptor, which comprises:

(a) contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing

DNA encoding a plurality of independent clones with a ligand, under conditions permitting activation of a mammalian G protein-coupled receptor;

(b) determining whether the ligand activates the cells expressing the plurality of independent clones and the chimeric G protein; and if so

(c) isolating the single clone which expresses the mammalian G protein-coupled receptor activated by the ligand, so as to thereby identify any clone included in the plurality of clones as encoding a mammalian G protein-coupled receptor.

The invention further provides a process of screening a plurality of independent clones not known to include a clone encoding a mammalian G protein-coupled receptor, to identify and isolate a clone encoding a mammalian G protein-coupled receptor, which comprises:

(a) contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a plurality of independent clones with a ligand, under conditions permitting specific binding to a mammalian G protein-coupled receptor;

(b) determining whether the ligand specifically binds to the cells expressing the plurality of independent clones and the chimeric G protein; and if so

(c) isolating the single clone which expresses the mammalian G protein-coupled receptor which specifically binds to the ligand, so as to thereby identify any clone included in the plurality of clones as encoding a mammalian G protein-coupled receptor.

In one embodiment of the aforementioned processes, the DNA encoding the plurality of independent clones is endogenous to the cell. In another embodiment, the DNA encoding the plurality of independent clones is transfected into the cell.

In one embodiment of the aforementioned processes, the chimeric G protein comprises an invertebrate Gαq G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate G protein beginning with the C-terminal amino acid of such vertebrate G protein, wherein such number equals the number of amino acids deleted. In another embodiment, the chimeric G protein comprises an invertebrate Gαq G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate Gαz protein beginning with the C-terminal amino acid of such vertebrate Gαz protein, wherein such number equals the number of amino acids deleted. In another embodiment, the chimeric G protein comprises an invertebrate Gαq G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been

deleted and replaced by a number of contiguous amino acids present in a vertebrate Gαs protein beginning with the C-terminal amino acid of such vertebrate Gαs protein, wherein such number equals the number of amino acids deleted. In another embodiment, the chimeric G protein comprises an invertebrate Gαq G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate Gαi3 protein beginning with the C-terminal amino acid of such vertebrate Gαi3 protein, wherein such number equals the number of amino acids deleted.

In another embodiment, the chimeric G protein comprises an *Caenorhabditis elegans* Gαq G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate G protein beginning with the C-terminal amino acid of such vertebrate G protein, wherein such number equals the number of amino acids deleted. In other embodiments, the chimeric G protein comprises a *Drosophila melanogaster* Gαq G protein, a *Limulus polyphemus* Gαq G protein, a *Patinopecten yessoensis* Gαq G protein, a *Loligo forbesi* Gαq G protein, a *Homarus americanus* Gαq G protein, a *Lymnaea stagnalis* Gαq G protein, a *Geodia cydonium* Gαq G protein, or a *Dictyostelium discoideum* $G\alpha_4$ G protein, from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate G protein beginning with the C-terminal amino acid of

such vertebrate G protein, wherein such number equals the number of amino acids deleted. In other embodiments, the chimeric G protein has an amino acid sequence substantially the same as the amino acid sequence shown in (a) Figure 2, *C. elegans* $G\alpha_{q/z5}$ (SEQ ID NO: 1); (b) Figure 2, *C. elegans* $G\alpha_{q/z9}$ (SEQ ID NO: 2); (c) Figure 2, *C. elegans* $G\alpha_{q/s9}$ (SEQ ID NO: 3); (d) Figure 2, *C. elegans* $G\alpha_{q/s21}$ (SEQ ID NO: 4); (e) Figure 2, *C. elegans* $G\alpha_{q/i3(5)}$ (SEQ ID NO: 5); or (f) Figure 2, *D. melanogaster* $G\alpha_{q/z5}$ (SEQ ID NO: 41).

In one embodiment of the aforementioned processes, the cell is an insect cell. In another embodiment, the cell is a mammalian cell. In one embodiment, the mammalian cell is nonneuronal in origin. In further embodiments, the nonneuronal cell is a COS-7 cell, 293 human embryonic kidney cell, a CHO cell, a NIH-3T3 cell, a mouse Y1 cell, or a LM(tk-) cell.

The invention provides a process for making a composition of matter which specifically binds to a mammalian G protein-coupled receptor which comprises identifying a chemical compound using any of the aforementioned processes and then synthesizing the chemical compound or a novel structural and functional analog or homolog thereof. The invention also provides a process for preparing a composition which comprises admixing a carrier and a pharmaceutically effective amount of a chemical compound identified by any of the aforementioned processes or a novel structural and functional analog or homolog thereof.

GPCRs that can be used with the invention include, but are not limited to, neuropeptide FF receptors, e.g., human NPFF1 (ATCC Accession number 203605) and human NPFF2 (ATCC Accession number 203255). Plasmid pcDNA3.1-hNPFF1 and plasmid pCDNA3.1-hNPFF2b were deposited on January 21, 1999 and September 22, 1998, respectively, with the American Type Culture Collection (ATCC), 10801 University Blvd., Manassas, Virginia 20110-2209, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and were accorded ATCC Accession Nos. 203605 and 203255, respectively.

Further GPCRs that can be used with the invention include, but are not limited to, serotonin receptors, e.g., human 5HT1D (U.S. Patent No. 5,155,218, the disclosure of which is hereby incorporated by reference in its entirety into this application), rabbit 5HT1D (Harwood, G. et al., 1995), human 5HT7 (ATCC Accession number 75332), human 5HT1E (U.S. Patent No. 5,476,782, the disclosure of which is hereby incorporated by reference in its entirety into this application), human 5HT1F (U.S. Patent No. 5,360,735, the disclosure of which is hereby incorporated by reference in its entirety into this application), human 5HT5A (Plassat et al., 1992), human 5HT5B (Matthes et al., 1993), human 5HT1B (U.S. Patent No. 5,155,218, the disclosure of which is hereby incorporated by reference in its entirety into this application), human 5HT4 (U.S. Patent No. 5,766,879, the disclosure of which is hereby incorporated by reference in its entirety into this application), human 5HT6 (Kohen et al., 1996), and human 5HT1A (Kobilka et al., 1987). Plasmid pcEXV-$5HT_{4B}$ was deposited on October 20, 1992 with the

American Type Culture Collection (ATCC), 10801 University Blvd., Manassas, Virginia 20110-2209, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and was accorded ATCC Accession No. 75332.

Further GPCRs that can be used with the invention include, but are not limited to, dopamine receptors, e.g., human D1, human D2, human D3, and human D5 (U.S. Patent No. 5,780,485, the disclosure of which is hereby incorporated by reference in its entirety into this application), and alpha adrenergic receptors, e.g., human α1A adr, human α2C adr, human α2B adr, human α2A adr (U.S. Patent No. 5,780,485, the disclosure of which is hereby incorporated by reference in its entirety into this application), and human β2 adr (Dixon et al., 1986).

Further GPCRs that can be used with the invention include, but are not limited to, galanin receptors, e.g., human GALR1 (Habert-Ortoli et al., 1994), rat GALR1 (Burgevin et al., 1995), human GALR2 (ATCC Accession No. 97851), rat GALR2 (ATCC Accession No. 97426), human GALR3 (ATCC Accession No. 97827), and rat GALR3 (ATCC Accession No. 97826). Plasmids pEXJ-hGalR3 and pEXJ-rGALR3T were deposited on December 17, 1996, with the American Type Culture Collection (ATCC), 10801 University Blvd., Manassas, Virginia 20110-2209, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and were accorded ATCC Accession Nos. 97827 and 97826, respectively. Plasmids BO39 and K985 were deposited on January 15, 1997 and January 24, 1996, respectively, with the American Type

Culture Collection (ATCC), 10801 University Blvd., Manassas, Virginia 20110-2209, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and were accorded ATCC Accession Nos. 97851 and 97426, respectively.

Further GPCRs that can be used with the invention include, but are not limited to, neuropeptide Y receptors, e.g., human Y1 (Larhammar et al., 1992), rat Y1 (Eva et al., 1990), human Y2 (U.S. Patent No. 5,545,549, the disclosure of which is hereby incorporated by reference in its entirety into this application), human Y4 (U.S. Patent No. 5,516,653, the disclosure of which is hereby incorporated by reference in its entirety into this application), rat Y4 (ATCC Accession No. 75984), human Y5 (U.S. Patent No. 5,602,024, the disclosure of which is hereby incorporated by reference in its entirety into this application), and rat Y5 (U.S. Patent No. 5,602,024, the disclosure of which is hereby incorporated by reference in its entirety into this application). Plasmid pcEXV-rY4 was deposited on December 21, 1994 with the American Type Culture Collection (ATCC), 10801 University Blvd., Manassas, Virginia 20110-2209, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and were accorded ATCC Accession No. 75984.

Further GPCRs that can be used with the invention include, but are not limited to, neurotensin receptors, e.g., rat NTR1 (Tanaka et al., 1990); glucagon-like peptide receptors, e.g., human GLP-1 (Dillon et al., 1993); kappa opioid receptors, e.g.,

human kappa (Mansson et al., 1994); and melanin concentrating hormone receptors, e.g., human MCH (ATCC Accession No. 203197). Plasmid pEXJ.HR-TL231 was deposited on September 17, 1998 with the American Type Culture Collection (ATCC), 10801 University Blvd., Manassas, Virginia 20110-2209, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and were accorded ATCC Accession No. 203197.

The invention will be better understood from the Experimental Details which follow. However, one skilled in the art will readily appreciate that the specific methods and results discussed are merely illustrative of the invention as described more fully in the claims which follow thereafter.

Experimental Details

Materials and Methods

Cloning of the gene encoding *C. elegans* $G\alpha_q$

The gene for wild-type *C. elegans* $G\alpha_q$ was obtained by PCR amplification of a mixed stage *C. elegans* cDNA library (Stratagene, #937006) with the primers RP65 and RP66 (Table 1). The resulting product was cloned into the vector pcDNA 3.1 Zeo (Invitrogen) at the KpnI and XbaI sites. DNA sequence analysis demonstrated that the clone designated R48 was identical to that of the *C. elegans* $G\alpha_q$ gene deposited in Genbank (accession number AF003739).

Cloning of the gene encoding *D. melanogaster* $G\alpha_q$

The gene for wild-type *D. melanogaster* $G\alpha_q$ (isoform 3) was obtained by PCR amplification of *D. melanogaster* cDNA using primers RP203 and RP204 (Table 1). The resulting product was cloned into pcDNA3.1 (Invitrogen) at the KpnI and EcoRI sites. DNA sequence analysis demonstrated that the clone designated R129 encoded a protein identical to that of the *D. melanogaster* $G\alpha_{q3}$ gene deposited in Genbank (accession number P54400).

Cloning of genes encoding human $G\alpha_q$

The sequence of human $G\alpha_q$ was confirmed by automated sequence analysis. Except for the substitution of a single amino acid at position 171 (Ala → Ser) in a highly non-conserved region of the protein, the deduced amino acid sequence is identical to that of Accession Number L76256. This sequence was used to generate the various human chimerae described throughout this application, except as noted in Table

9. A second human $G\alpha_q$ clone was obtained using standard PCR-based techniques that has a sequence identical to Genbank entry L76256. As expected, chimerae utilizing these two independently derived human $G\alpha_q$ sequences were found to be functionally indistinguishable in parallel assays (Table 9), using the dopamine D1 receptor as an example.

Construction of G protein cDNAs with chimeric 3′ ends

Most of the chimeric G protein cDNAs were made by a PCR approach (Table 2). In each case, the designated primers were used to amplify the 3′ end of the appropriate template to generate a chimeric PCR product. This product was then subcloned back into wild-type human, *D. melanogaster*, or *C. elegans* $G\alpha_q$, as appropriate, to generate a full-length chimeric gene. All PCR derived sequences were verified by sequence analysis. Two chimeras (Table 3) were constructed using the QuikChange site-directed mutagenesis kit (Stratagene, #200518). For these clones, the sequence of the entire coding region was verified. Examples of chimeric G proteins used in the present application are depicted in Figure 2.

TABLE 1. Primer sequences used in the preparation of chimeric G protein genes

PRIMER	SEQUENCE
MJ177	5′ GAATATGATGGACCCCAGAGAGATG 3′ (SEQ ID NO: 19)
MJ178	5′ GATCCTCGAGTTAGCACAGTCCGATGTACTTCAGGTTC AACTGGAGGATGGT 3′ (SEQ ID NO: 20)
MJ180	5′ GATCCTCGAGTTAGTACAGTCCGCATCCCTTCAGGTTCA ACTGGAGGATGGT 3′ (SEQ ID NO: 21)
MJ193	5′ GATCCTCGAGTTAGTAAAGCCCACATTCCTTCAGGTTC AACTGGAGGATGGT 3′ (SEQ ID NO: 22)

MJ194	5′ GATCCTCGAGTTAGAGCAGCTCGTATTGCTTCAGGTTCA ACTGGAGGATGGT 3′ (SEQ ID NO: 23)
MJ197	5′ GGAAAAAAGCGGCCGCTTAAAACAGTCCGCAGTCC TTCAGGTTCAACTGGAGGATGGT 3′ (SEQ ID NO: 24)
RP65	5′ GGGGTACCGCCGCCATGGCCTGCTGTTTATCC 3′ (SEQ ID NO: 25)
RP66	5′ GCTCTAGATTACACCAAGTTGTACTCCTTCAGATT 3′ (SEQ ID NO: 26)
RP80	5′ CTCTCCGATCTCCGACGGCTG 3′ (SEQ ID NO: 27)
RP83	5′ TTCTACAGCATAATCTGAAGTATATCGGTTTGTGTTAATCT AGAGGGCCCGTTTAAACCCGCTG 3′ (SEQ ID NO: 28)
RP84	5′ CAGCGGGTTTAAACGGGCCCTCTAGATTAACACAAACCGAT ATACTTCAGATTATGCTGTAGAA 3′ (SEQ ID NO: 29)
RP85	5′ CAGCATAATCTGAAGGAGTGTGGATTGTACTAATCTAGA GGGCCCG 3′ (SEQ ID NO: 30)
RP86	5′ CGGGCCCTCTAGATTAGTACAATCCACACTCCTTCAG ATTATGCTG 3′ (SEQ ID NO: 31)
RP116	5′ GGAAAAAAGCGGCCGCTTAGAGCAGCTCGTATTGC CTCAGGTGCATCTGGAGGATGGTGTCCTTGACGG 3′ (SEQ ID NO: 32)
RP142	5′ GCTCTAGATTAGAGCAGCTCGTATTGCCTCAGGTGCATCTG TAGAATTGTGTCTTTGACGGCG 3′ (SEQ ID NO: 33)
RP168	5′ GCTCTAGATTAACATAGCCCTATGTATTTTAGATTATTCTG TAGAATTGTGTCTTTGACGGCG 3′ (SEQ ID NO: 34)
RP177	5′ GCTCTAGATTAGAGCAGCTCGTATTGCCTCAGGTGCATACG TTGAATAATGTCACGACAGTCATTAAAAACACGCCGAATGT TTTCCGTATCAGTCGC 3′ (SEQ ID NO: 35)

RP203	5' CGGGGTACCCCGGTTAGCATGGAGTGCTGTTTATCG 3' (SEQ ID NO: 42)
RP204	5' CCGGAATTCCGGTTAGACCAAATTATATTCCTTAAGGTTC 3' (SEQ ID NO: 43)
RP218	5' GAGCATCGATTACGAGACCGTTACC 3' (SEQ ID NO: 44)
RP219	5' CGGAATTCTTAGCACAGTCCGATGTACTTAAGGTTCGATTG CAGAATTGTGTC 3' (SEQ ID NO: 45)

TABLE 2. Primer pairs used to generate chimeric genes by PCR

CHIMERA	PCR TEMPLATE	PRIMERS
Human $G\alpha_{q/z5}$	$hG\alpha_q$	MJ177 / MJ178
Human $G\alpha_{q/i2(5)}$	$hG\alpha_q$	MJ177 / MJ197
Human $G\alpha_{q/i3(5)}$	$hG\alpha_q$	MJ177 / MJ193
Human $G\alpha_{q/o5}$	$hG\alpha_q$	MJ177 / MJ180
Human $G\alpha_{q/s5}$	$hG\alpha_q$	MJ177 / MJ194
Human $G\alpha_{q/s9}$	$hG\alpha_q$	MJ177 / RP116
C.elegans $G\alpha_{q/s9}$	R48	RP80 / RP142
C. elegans $G\alpha_{q/s21}$	R48	RP80 / RP177
C. elegans $G\alpha_{q/z9}$	R48	RP80 / RP168
D. melanogaster $G\alpha_{q/z}$	R129	RP218/ RP219

TABLE 3. Primer pairs used to generate chimeric genes using mutagenesis

CHIMERA	TEMPLATE	PRIMERS
C.elegans $G\alpha_{q/i3(5)}$	R48	RP85 / RP86
C.elegans $G\alpha_{q/z5}$	R48	RP83 / RP84

General methods of transfecting cells

Methods of transfecting cells, e.g. mammalian cells, with such nucleic acid encoding a GPCR to obtain cells in which the GPCR is expressed on the surface of the cell are well known in the art. (See, for example, U.S. Patent Nos. 5,053,337; 5,155,218; 5,360,735; 5,472,866; 5,476,782; 5,516,653; 5,545,549; 5,556,753; 5,595,880; 5,602,024; 5,639,652; 5,652,113; 5,661,024; 5,766,879; 5,786,155; and 5,786,157, the disclosures of which are hereby incorporated by reference in their entireties into this application.) The cells may be additionally transfected with nucleic acid encoding chimeric G proteins to obtain cells in which both the GPCR and the chimeric G proteins are expressed in the cell.

Such transfected cells may also be used to test compounds and screen compound libraries to obtain compounds which bind receptors as well as compounds which activate or inhibit activation of functional responses in such cells, and therefore are likely to do so in vivo. (See, for example, U.S. Patent Nos. 5,053,337; 5,155,218; 5,360,735; 5,472,866; 5,476,782; 5,516,653; 5,545,549; 5,556,753; 5,595,880; 5,602,024; 5,639,652; 5,652,113; 5,661,024; 5,766,879; 5,786,155; and 5,786,157, the disclosures of which are hereby incorporated by reference in their entireties into this application.)

Host cells

A broad variety of host cells can be used to study heterologously expressed proteins. These cells include but are not limited to mammalian cell lines such as; Cos-7, CHO, LM(*tk*$^-$), HEK293, etc.; insect cell lines such as; Sf9, Sf21, etc.; amphibian cells

such as *Xenopus oocytes*; assorted yeast strains; assorted bacterial cell strains; and others. Culture conditions for each of these cell types is specific and is known to those familiar with the art.

Transient expression

DNA encoding proteins to be studied can be transiently expressed in a variety of mammalian, insect, amphibian, yeast, bacterial and other cells lines by several transfection methods including but not limited to: calcium phosphate-mediated, DEAE-dextran mediated; liposomal-mediated, viral-mediated, electroporation-mediated, and microinjection delivery. Each of these methods may require optimization of assorted experimental parameters depending on the DNA, cell line, and the type of assay to be subsequently employed.

Stable expression

Heterologous DNA can be stably incorporated into host cells, causing the cell to perpetually express a foreign protein. Methods for the delivery of the DNA into the cell are similar to those described above for transient expression but require the co-transfection of an ancillary gene to confer drug resistance on the targeted host cell. The ensuing drug resistance can be exploited to select and maintain cells that have taken up the DNA. An assortment of resistance genes are available including but not restricted to neomycin, kanamycin, and hygromycin.

Mammalian cell tissue culture and transfection.

COS-7 cells were cultured in 225 cm^2 flasks in Dulbecco's Modified Eagle Medium (DMEM) with supplements (10% bovine calf serum, 4 mM glutamine,

100 units/ml penicillin and 100 µg/ml streptomycin) at 37°C, 5% CO_2. Stock plates of COS-7 cells were trypsinized and split 1:6 every 3-4 days.

GPCR and chimeric G protein cDNAs were transiently transfected into COS-7 cells in 150 cm^2 flasks by the DEAE-dextran method (Cullen, 1987), using a total of 20 µg of DNA/ ~ 7 x 10^6 cells. For evaluating the function of a single chimeric G protein, the standard cDNA transfection ratio was 1:1 (10 µg GPCR cDNA and 10 µg chimeric G protein cDNA). For evaluating the function of a mixture of chimeric G proteins, the standard cDNA transfection ratio was 8:1:1 (16 µg GPCR cDNA, 2 µg $G\alpha_{q/z}$ cDNA, 2 µg $G\alpha_{q/s}$ cDNA).

Membrane preparations

Cell membranes expressing the heterologously expressed proteins of this invention are useful for certain types of assays including but not restricted to ligand binding assays, GTPγS binding assays, and others. The specifics of preparing such cell membranes may in some cases be determined by the nature of the ensuing assay but typically involve harvesting whole cells and disrupting the cell pellet by sonication in ice cold buffer (e.g. 20 mM Tris-HCl, 5 mM EDTA, pH 7.4). The resulting crude cell lysate is cleared of cell debris by low speed centrifugation at 200xg for 5 min at 4°C. The cleared supernatant is then centrifuged at 40,000xg for 20 min at 4°C, and the resulting membrane pellet is washed by suspending in ice cold buffer and repeating the high speed centrifugation step. The final washed membrane pellet is resuspended in assay buffer. Protein concentrations are determined by the method of Bradford (1976) using bovine serum albumin

as a standard. The membranes may be used immediately or frozen for later use.

Generation of baculovirus

The coding region of DNA encoding the human receptor and the chimeric G protein disclosed herein may be separately subcloned into pBlueBacIII into existing restriction sites or sites engineered into sequences 5' and 3' to the coding region of the polypeptides. To generate baculovirus, 0.5 µg of viral DNA (BaculoGold) and 3 µg of DNA construct encoding a polypeptide may be co-transfected into 2 x 10^6 *Spodoptera frugiperda* insect Sf9 cells by the calcium phosphate co-precipitation method, as outlined by Pharmingen (in "Baculovirus Expression Vector System: Procedures and Methods Manual"). The cells are then incubated for 5 days at 27°C.

The supernatant of the co-transfection plate may be collected by centrifugation and the recombinant virus plaque purified. The procedure to infect cells with virus, to prepare stocks of virus and to titer the virus stocks are as described in Pharmingen's manual.

Binding assays

Labeled ligands are placed in contact with either membrane preparations or intact cells expressing the chimeric G protein and receptor of interest in multi-well microtiter plates, together with unlabeled compounds, and binding buffer. Binding reaction mixtures are incubated for times and temperatures determined to be optimal in separate equilibrium binding assays. The reaction is stopped by filtration through GF/B filters, using a cell harvester, or by directly measuring the bound ligand. If the ligand was labeled with a radioactive isotope

such as ^{3}H, ^{14}C, ^{125}I, ^{35}S, ^{32}P, ^{33}P, etc., the bound ligand may be detected by using liquid scintillation counting, scintillation proximity, or any other method of detection for radioactive isotopes. If the ligand was labeled with a fluorescent compound, the bound labeled ligand may be measured by methods such as, but not restricted to, fluorescence intensity, time resolved fluorescence, fluorescence polarization, fluorescence transfer, or fluorescence correlation spectroscopy. In this manner, agonist or antagonist compounds that bind to the receptor may be identified as they inhibit the binding of the labeled ligand to the membrane protein or intact cells expressing the said receptor. Non-specific binding is defined as the amount of labeled ligand remaining after incubation of membrane protein in the presence of a high concentration (e.g., 100-1000 X K_D) of unlabeled ligand. In equilibrium saturation binding assays membrane preparations or intact cells transfected with the chimeric G protein and GPCR are incubated in the presence of increasing concentrations of the labeled compound to determine the binding affinity of the labeled ligand. The binding affinities of unlabeled compounds may be determined in equilibrium competition binding assays, using a fixed concentration of labeled compound in the presence of varying concentrations of the displacing ligands.

Functional assays

Cells expressing the chimeric G protein DNA of this invention and a GPCR may be used to screen for ligands to the GPCR using functional assays. Once a ligand is identified, the same assays may be used to identify agonists or antagonists of the GPCR that may be employed for a variety of therapeutic purposes.

It is well known to those in the art that the over-expression of a G protein-coupled receptor can result in the constitutive activation of intracellular signaling pathways. In the same manner, over-expression of an orphan receptor and a chimeric G protein in any cell line as described above, can result in the activation of the functional responses described below, and any of the assays herein described can be used to screen for both agonist and antagonist ligands of the orphan receptor.

A wide spectrum of assays can be employed to screen for the presence of orphan receptor ligands or to identify agonists or antagonists of a characterized GPCR. These assays range from traditional measurements of total inositol phosphate accumulation, cAMP levels, intracellular calcium mobilization, and potassium currents, for example; to systems measuring these same second messengers, but which have been modified or adapted to be of higher throughput, more generic, and more sensitive; to cell based assays reporting more general cellular events resulting from receptor activation such as metabolic changes, differentiation, cell division/proliferation. Description of several such assays follow.

Cyclic AMP (cAMP) assay

Elevation of intracellular Ca^{++} can modulate the activity of adenylyl cyclases via Ca^{++}-dependent calmodulin (Sunahara et al., 1996). The receptor-mediated stimulation or inhibition of cyclic AMP (cAMP) formation may be assayed in cells expressing a GPCR and chimeric G protein. Cells are plated in 96-well plates or other vessels and preincubated in a buffer such as HEPES buffered saline (NaCl (150 mM),

$CaCl_2$ (1 mM), KCl (5 mM), glucose (10 mM)) supplemented with a phosphodiesterase inhibitor such as 5mM theophylline, with or without protease inhibitor cocktail for 20 min at 37°C, in 5% CO_2. A typical inhibitor cocktail contains 2 μg/ml aprotinin, 0.5 mg/ml leupeptin, and 10 μg/ml phosphoramidon. Test compounds are added with or without 10 mM forskolin and incubated for an additional 10 min at 37°C. The medium is then aspirated and the reaction stopped by the addition of 100 mM HCl or other methods. The plates are stored at 4°C for 15 min, and the cAMP content in the stopping solution is measured by radioimmunoassay. Radioactivity may be quantified using a gamma counter equipped with data reduction software. Specific modifications may be performed to optimize the assay for the GPCR or to alter the detection method of cAMP.

Arachidonic acid release assays

Cells expressing a GPCR and chimeric G protein are seeded into 96 well plates or other vessels and grown for 3 days in medium with supplements. ^{3}H-arachidonic acid (specific activity = 0.75 μCi/ml) is delivered as a 100 μL aliquot to each well and samples are incubated at 37° C, 5% CO_2 for 18 hours. The labeled cells are washed three times with medium. The wells are then filled with medium and the assay is initiated with the addition of test compounds or buffer in a total volume of 250 μL. Cells are incubated for 30 min at 37°C, 5% CO_2. Supernatants are transferred to a microtiter plate and evaporated to dryness at 75°C in a vacuum oven. Samples are then dissolved and resuspended in 25 μL distilled water. Scintillant (300 μL) is added to each well

and samples are counted for ^3H in a Trilux plate reader. Data are analyzed using nonlinear regression and statistical techniques available in the GraphPAD Prism package (San Diego, CA).

Intracellular calcium mobilization assay

Twenty four hours after transient transfection, COS-7 cells were seeded into 96-well black wall microtiter plates coated with poly-D-lysine for assay the following day. Just prior to assay, culture medium was aspirated and cells were dye-loaded with 4 μM Fluo-3/ 0.01% pluronic acid in assay buffer composed of Hank's Balanced Salt Solution (138 mM NaCl, 5 mM KCl, 1.3 mM $CaCl_2$, 0.5 mM $MgCl_2$, 0.4 mM $MgSO_4$, 0.3 mM KH_2PO_4, 0.3 mM Na_2HPO_4, 5.6 mM glucose) plus 20 mM HEPES (pH 7.4), 0.1% BSA and 2.5 mM probenicid (100 μl/well) for 1 hour in 5% CO_2 at 37 °C. After excess dye was discarded, cells were washed in assay buffer and layered with a final volume equal to 100 μl/well. Basal fluorescence was monitored in a fluorometric imaging plate reader (FLIPR™, Molecular Devices) with an excitation wavelength of 488 nm and an emission range of 500 to 560 nm. Laser excitation energy was adjusted so that basal fluorescence readings were approximately 10,000 relative fluorescent units. Cells were stimulated with agonists diluted in assay buffer (50 μl), and relative fluorescent units were measured at defined intervals (exposure = 0.4 sec) over a 3 min period at room temperature. Maximum change in fluorescence was calculated for each well. Concentration-response curves derived from the maximum change in fluorescence were analyzed by nonlinear regression (Hill equation).

Alternatively, intracellular free calcium concentration may be measured by microspectrofluorimetry using the fluorescent indicator dye Fura-2/AM (Bush et al, 1991). Cells expressing the receptor and chimeric G protein are seeded onto a 35 mm culture dish containing a glass coverslip insert and allowed to adhere overnight. Cells are then washed with HBS and loaded with 100 μL of Fura-2/AM (10 μM) for 20 to 40 min. After washing with HBS to remove the Fura-2/AM solution, cells are equilibrated in HBS for 10 to 20 min. Cells are then visualized under the 40X objective of a Leitz Fluovert FS microscope and fluorescence emission is determined at 510 nM with excitation wavelengths alternating between 340 nM and 380 nM. Raw fluorescence data are converted to calcium concentrations using standard calcium concentration curves and software analysis techniques.

Alternative calcium-sensitive indicators may be used. Preferred examples of these are: aequorin, Fluo-3, Fluo-4, Fluo-5, Calcium Green-1, Oregon Green, and 488 BAPTA. After activation of the receptors with agonist ligands the emission elicited by the change of intracellular calcium concentration can be measured by a luminometer or a fluorescence imager; a preferred example of this is the fluorescence imager plate reader (FLIPR™) as described above. Antagonist ligands are identified by the inhibition of the signal elicited by agonist ligands.

Inositol phosphate assay

Receptor mediated activation of the inositol phosphate (IP) second messenger pathways may be assessed by radiometric or other measurement of IP products. For example, in a 96 well microplate format

assay, cells are plated at a density of 70,000 cells per well and allowed to incubate for 24 hours. The cells are then labeled with 0.5 μCi [^{3}H]myo-inositol overnight at 37^{0}C, 5% CO_2. Immediately before the assay, the medium is removed and replaced with 90 μL of PBS containing 10 mM LiCl. The plates are then incubated for 15 min at 37^{0}C, 5% CO_2. Following the incubation, the cells are challenged with agonist (10 μl/well; 10x concentration) for 30 min at 37^{0}C, 5% CO_2. The challenge is terminated by the addition of 100 μL of 50% v/v trichloroacetic acid, followed by incubation at 4^{0}C for greater than 30 minutes. Total IPs are isolated from the lysate by ion exchange chromatography. Briefly, the lysed contents of the wells are transferred to a Multiscreen HV filter plate (Millipore) containing Dowex AG1-X8 (200-400 mesh, formate form). The filter plates are prepared adding 100 μL of Dowex AG1-X8 suspension (50% v/v, water: resin) to each well. The filter plates are placed on a vacuum manifold to wash or elute the resin bed. Each well is first washed 2 times with 200 μl of 5 mM myo-inositol. Total [^{3}H]inositol phosphates are eluted with 75 μl of 1.2 M ammonium formate/0.1 M formic acid solution into 96-well plates. 200 μL of scintillation cocktail is added to each well and the radioactivity is determined by liquid scintillation counting.

GTPγS binding assay

Membranes from cells expressing a GPCR and a chimeric G protein are suspended in assay buffer (e.g., 50 mM Tris, 100 mM NaCl, 5 mM $MgCl_2$, 10 μM GDP, pH 7.4) with or without protease inhibitors (e.g., 0.1% bacitracin). Membranes are incubated on ice for 20 minutes, transferred to a 96-well Millipore

microtiter GF/C filter plate and mixed with GTPγ^{35}S (e.g., 250,000 cpm/sample, specific activity ~1000 Ci/mmol) plus or minus unlabeled GTPγS (final concentration = 100 μM). The final membrane protein concentration is approximately 20 μg/ml. Samples are incubated in the presence or absence of test compounds for 30 minutes at room temperature, then filtered on a Millipore vacuum manifold and washed three times with cold (4°C) assay buffer. Samples collected in the filter plate are treated with scintillant and counted for ^{35}S in a Trilux (Wallac) liquid scintillation counter. It is expected that optimal results are obtained when the receptor membrane preparation is derived from an appropriately engineered heterologous expression system, i.e., an expression system resulting in high levels of expression of the receptor and/or expressing G proteins having high turnover rates (for the exchange of GDP for GTP). GTPγS assays are well-known to those skilled in the art, and it is contemplated that variations on the method described above, such as are described by Tian et al. (1994) or Lazareno and Birdsall (1993), may be used.

MAP kinase assay

MAP kinase (mitogen activated kinase) may be monitored to evaluate receptor activation. MAP kinase is activated by multiple pathways in the cell. A primary mode of activation involves the ras/raf/MEK/MAP kinase pathway. Growth factor (tyrosine kinase) receptors feed into this pathway via SHC/Grb-2/SOS/ras. Gi coupled receptors are also known to activate ras and subsequently produce an activation of MAP kinase. Receptors that activate phospholipase C (such as Gq /G11 -coupled) produce

diacylglycerol (DAG) as a consequence of phosphatidyl inositol hydrolysis. DAG activates protein kinase C which in turn phosphorylates MAP kinase.

MAP kinase activation can be detected by several approaches. One approach is based on an evaluation of the phosphorylation state, either unphosphorylated (inactive) or phosphorylated (active). The phosphorylated protein has a slower mobility in SDS-PAGE and can therefore be compared with the unstimulated protein using Western blotting. Alternatively, antibodies specific for the phosphorylated protein are available (New England Biolabs) which can be used to detect an increase in the phosphorylated kinase. In either method, cells are stimulated with the test compound and then extracted with Laemmli buffer. The soluble fraction is applied to an SDS-PAGE gel and proteins are transferred electrophoretically to nitrocellulose or Immobilon. Immunoreactive bands are detected by standard Western blotting technique. Visible or chemiluminescent signals are recorded on film and may be quantified by densitometry.

Another approach is based on evaluation of the MAP kinase activity via a phosphorylation assay. Cells are stimulated with the test compound and a soluble extract is prepared. The extract is incubated at 30°C for 10 min with gamma-^{32}P-ATP, an ATP regenerating system, and a specific substrate for MAP kinase such as phosphorylated heat and acid stable protein regulated by insulin, or PHAS-I. The reaction is terminated by the addition of H_3PO_4 and samples are transferred to ice. An aliquot is spotted onto Whatman P81 chromatography paper, which retains the phosphorylated protein. The

chromatography paper is washed and counted for ^{32}P in a liquid scintillation counter. Alternatively, the cell extract is incubated with gamma-^{32}P-ATP, an ATP regenerating system, and biotinylated myelin basic protein bound by streptavidin to a filter support. The myelin basic protein is a substrate for activated MAP kinase. The phosphorylation reaction is carried out for 10 min at 30°C. The extract can then by aspirated through the filter, which retains the phosphorylated myelin basic protein. The filter is washed and counted for ^{32}P by liquid scintillation counting.

Cell proliferation assay

Receptor activation of a GPCR may lead to a mitogenic or proliferative response which can be monitored *via* ^{3}H-thymidine uptake. When cultured cells are incubated with ^{3}H-thymidine, the thymidine translocates into the nuclei where it is phosphorylated to thymidine triphosphate. The nucleotide triphosphate is then incorporated into the cellular DNA at a rate that is proportional to the rate of cell growth. Typically, cells are grown in culture for 1-3 days. Cells are forced into quiescence by the removal of serum for 24 hrs. A mitogenic agent is then added to the media. Twenty-four hrs later, the cells are incubated with ^{3}H-thymidine at specific activities ranging from 1 to 10 μCi/ml for 2-6 hrs. Harvesting procedures may involve trypsinization and trapping of cells by filtration over GF/C filters with or without a prior incubation in TCA to extract soluble thymidine. The filters are processed with scintillant and counted for ^{3}H by liquid scintillation counting. Alternatively, adherent cells are fixed in MeOH or TCA, washed in water, and solubilized in 0.05%

deoxycholate/0.1 N NaOH. The soluble extract is transferred to scintillation vials and counted for ^3H by liquid scintillation counting.

Alternatively, cell proliferation can be assayed by measuring the expression of an endogenous or heterologous gene product, expressed by the cell line used to transfect the GPCR, which can be detected by methods such as, but not limited to, fluorescence intensity, enzymatic activity, immunoreactivity, DNA hybridization, polymerase chain reaction, etc.

Reporter gene assays

The chimeric Gα subunits described in this application can be used in conjunction with any number of $G\alpha_q$-linked transcriptional assays to include GPCRs that do not normally use $G\alpha_q$ as their native signaling pathway. This application could include, but is not limited to, the use of Gα chimeras to link activation of any GPCR to a fluorescent signal generated via a reporter enzyme such as β-lactamase placed under the transcriptional regulation of NFAT, SRE, CRE, AP-1, TRE IRE or other specific DNA regulatory elements or promoters (Naylor, 1999).

Methods for recording currents in *Xenopus* oocytes

Oocytes were harvested from *Xenopus laevis* and injected with mRNA transcripts as previously described (Quick and Lester, 1994; Smith et al., 1997). Receptor and chimeric G protein α subunit RNA transcripts were synthesized using the T7 polymerase ("Message Machine," Ambion) from linearized plasmids or PCR products containing the complete coding region of the genes. Oocytes were injected with 5-25 ng

synthetic receptor RNA and incubated for 3-8 days at 17 degrees. Three to eight hours prior to recording, oocytes were injected with 500 pg chimeric Gα subunit mRNA. Dual electrode voltage clamp (Axon Instruments Inc.) was performed using 3 M KCl-filled glass microelectrodes having resistances of 1-2 MOhm. Unless otherwise specified, oocytes were voltage clamped at a holding potential of -80 mV. During recordings, oocytes were bathed in continuously flowing (1-3 ml/min) medium containing 96 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, and 5 mM HEPES, pH 7.5 (ND96). Drugs were applied by local perfusion from a 10 µl glass capillary tube fixed at a distance of 0.5 mm from the oocyte. Experiments were carried out at room temperature. All values are expressed as mean ± standard error of the mean.

Beta-gamma-dependent signaling

Beta-gamma sub-units released from $G\alpha_q$ may interact with a variety of effectors, including phospholipase C beta, adenylate cyclase II and IV, ion channels (Kir 3.x family of K^+ channels, calcium channels), Ras and PI-3-gamma. Each of these may be monitored by specific read-outs known to those skilled in the art.

Expression cloning

The expression cloning strategy is a well-known method utilized to clone mammalian G protein-coupled receptors (Kluxen et al., 1992; Kiefer et al, 1992; Julius et al., 1988; US 5,545,549 and US 5,602,024, the disclosures of which are hereby incorporated by reference in their entireties into this application). A chimeric G protein of this invention may be utilized in expression cloning to facilitate identification of clones which encode mammalian G protein-coupled receptors. Cells, expressing the DNA

encoding numerous independent clones, may be transfected with and express DNA encoding a chimeric G protein of this invention. The presence of the chimeric G protein in the cells may facilitate ligand activation of or binding to a mammalian G protein-coupled receptor encoded by one of the independent clones which may be subsequently isolated.

Results and Discussion

Expression of *C. elegans* chimera in *Xenopus* oocytes

The chimeric Gα subunit consisting of $cG\alpha_{q/z5}$, wherein the C-terminal final 5 amino acids of $cG\alpha_q$ are replaced with those of $hG\alpha_z$ (Figure 2), was initially tested for expression and functional activity in *Xenopus* oocytes. Co-expression of $cG\alpha_{q/z5}$ with the NPFF1 receptor resulted in the appearance of large amplitude Cl^- currents following application of 1 μM NPFF (1258 ± 159 nA, n = 12). The currents stimulated by NPFF in oocytes expressing NPFF1 and $cG\alpha_{q/z5}$ were most likely mediated by the endogenous calcium-activated Cl^- channel (Gunderson et al., 1983), because they were blocked in oocytes injected with 50 nl of 10 mM EGTA (Figure 3). Chloride currents were also not observed from control oocytes expressing NPFF1 but lacking $cG\alpha_{q/z5}$ (n=15). In oocytes expressing NPFF1 and the human version of $G\alpha_{q/z5}$, response amplitudes (358 ± 67, n = 32) were about one third of those in oocytes expressing the *C. elegans* version of this chimera. Similar results were obtained with four additional GPCRs, GALR1, Y1, NPFF2, and 5HT1D, that are known to couple to either $G\alpha_i$ or $G\alpha_o$ (Table 4; Watling, 1998). The increase in response was 2-3 fold over currents recorded from oocytes expressing the human version of the chimera. The exception to this trend was coupling to the Y5 receptor, which was actually reduced with $cG\alpha_{q/z5}$. Extending the length of the $G\alpha_z$ portion of the C-terminal tail of $cG\alpha_q$ to 9 amino acids ($cG\alpha_{q/z9}$) did not further improve the amplitude of responses as compared to $cG\alpha_{q/z5}$ (Table 4).

TABLE 4. Response amplitudes (nA) in oocytes expressing various GPCRs and chimeric G proteins. Values are mean ± s.e.m (number of oocytes).

RECEPTOR	**CHIMERA**		
	hG$\alpha_{q/z5}$	**cG$\alpha_{q/z5}$**	**cG$\alpha_{q/z9}$**
Rabbit 5HT1D	90 ± 41 (14)	150 ± 105 (8)	34 ± 12 (9)
Rat GALR1	31 ± 16 (22)	91 ± 38 (15)	Not tested
Human NPFF1	358 ± 67 (32)	1258 ± 159 (12)	1449 ± 398 (5)
Human NPFF2	528 ± 99 (18)	1121 ± 261 (13)	Not tested
Rat Y1	841 ± 204 (19)	1549 ± 168 (13)	300 ± 177 (8)
Rat Y5	82 ± 43 (7)	0 ± 2 (8)	65 ± 34 (6)

Expression of chimeras in mammalian cells

To evaluate the utility of cG$\alpha_{q/z5}$ in mammalian cells, COS-7 cells were transiently transfected with either hG$\alpha_{q/z5}$ or cG$\alpha_{q/z5}$ plus a GPCR. In one example, cells transfected either with hG$\alpha_{q/z5}$ or cG$\alpha_{q/z5}$ plus the human D1 receptor, which is thought to be Gα_s- and G$\alpha_{i/o}$-coupling (Sidhu et al., 1991), were stimulated with dopamine at concentrations up to 100 µM and monitored for calcium mobilization (Figure 4). Whereas an agonist-induced response was undetectable with hG$\alpha_{q/z5}$ (n = 2), the cG$\alpha_{q/z5}$ construct supported an average maximum dopamine-stimulated signal of 12,120 relative fluorescence units (n = 2).

The data for human D1 clearly demonstrate that the probability of GPCR signal detection in mammalian cells can be enhanced by the use of a chimeric construct containing an invertebrate $G\alpha_q$ backbone (*C. elegans* $G\alpha_q$ in this case). To determine whether this effect extends to a broad range of GPCRs, $cG\alpha_{q/z5}$ was co-transfected into COS-7 cells with a panel of 36 different GPCRs, including $G\alpha_{i/o}$-, $G\alpha_s$-, and $G\alpha_q$-coupling receptors. Seventy eight percent (28/36) of the receptors generated positive signals (defined as > 500 fluorescence units) with $cG\alpha_{q/z5}$, compared to only 58% with $hG\alpha_{q/z5}$ (Table 5). Extending the $G\alpha_z$ tail length from 5 to 9 amino acids did not significantly change the detection rate (29/36 positive responses > 500 fluorescence units) but there was a trend, particularly among the most responsive receptors, toward a decreased maximal response. From these data, we can conclude that an invertebrate-based $G\alpha_{q/z}$ construct is optimal for detecting a broad range of GPCR, and we can identify $cG\alpha_{q/z5}$ as a preferred design.

TABLE 5. $G\alpha_{q/z5}$ chimeras and GPCR in COS-7 cells: agonist-induced response. Transfected cells were monitored for calcium mobilization in the FLIPR™ using the calcium sensitive dye Fluo-3. Maximum agonist concentrations were 100 μM for non-peptide ligands or 10 μM for peptide ligands, except for neurotensin (1 μM). Fluorescence data represent the mean from 2 or more experiments. h = human; m = mouse; r = rat; adr = adrenergic; DA = dopamine; GLP-1 = glucagon-like peptide; 5HT = serotonin; GAL = galanin; NE = norepinephrine; MCH = melanin-concentrating hormone; NPY = neuropeptide Y; PP =

pancreatic polypeptide; NPFF = neuropeptide FF; and NT = neurotensin

#	RECEPTOR	PROPOSED COUPLING	AGONIST	MAXIMAL SIGNAL (FLUORESCENCE UNITS)		
				$hG\alpha_{q/z5}$	$cG\alpha_{q/z5}$	$cG\alpha_{q/z9}$
1	h D3	$G\alpha_{i/o}$	DA	0	0	121
2	h GLP-1	$G\alpha_{s}$	GLP-1	287	131	135
3	h 5HT7	$G\alpha_{s}$	5HT	90	166	129
4	h 5HT1E	$G\alpha_{i/o}$	5HT	202	229	238
5	h 5HT1F	$G\alpha_{i/o}$	5HT	0	243	384
6	m 5HT5B	$G\alpha_{i/o}$	5HT	251	265	443
7	m 5HT5A	$G\alpha_{i/o}$	5HT	0	351	270
8	h 5HT1D	$G\alpha_{i/o}$	5HT	316	414	504
9	h D5	$G\alpha_{s}$, $G\alpha_{i/o}$	DA	782	657	797
10	h 5HT1B	$G\alpha_{i/o}$	5HT	405	929	1217
11	h 5HT4	$G\alpha_{s}$, $G\alpha_{q}$	5HT	2161	1011	1696
12	h 5HT6	$G\alpha_{s}$	5HT	210	1289	2287
13	h GALR3	$G\alpha_{i/o}$	GAL	804	1523	2050
14	h β2 adr	$G\alpha_{s}$, $G\alpha_{i/o}$	NE	128	1842	1697
15	h 5HT1A	$G\alpha_{i/o}$	5HT	478	1997	3139
16	r GALR3	$G\alpha_{i/o}$	GAL	2796	2298	2971
17	h MCH	$G\alpha_{q}$	MCH	783	2699	3332
18	r GALR1	$G\alpha_{i/o}$	GAL	82	3086	5947
19	r Y4	$G\alpha_{i/o}$	PP	4388	3662	2583
20	h α2C adr	$G\alpha_{i/o}$	NE	6106	4143	3874
21	r GALR2	$G\alpha_{q}$	GAL	4862	4198	4470
22	h α2B adr	$G\alpha_{i/o}$	NE	4515	4983	5138
23	h Y5	$G\alpha_{i/o}$	NPY	6407	5314	6680
24	h GALR2	$G\alpha_{q}$	GAL	5992	5470	4899
25	h kappa	$G\alpha_{i/o}$	U-69593	7864	5975	3472

#	RECEPTOR	PROPOSED COUPLING	AGONIST	MAXIMAL SIGNAL (FLUORESCENCE UNITS)		
				$hG\alpha_{q/z5}$	$cG\alpha_{q/z5}$	$cG\alpha_{q/z9}$
26	h NPFF1	$G\alpha_{i/o}$	NPFF	4717	6593	2966
27	h NPFF2	$G\alpha_{i/o}$	NPFF	19960	7566	4578
28	h α2A adr	$G\alpha_{i/o}$	NE	10933	7575	3040
29	h D2	$G\alpha_{i/o}$	DA	15579	7615	4305
30	h GALR1	$G\alpha_{i/o}$	GAL	4061	7648	8489
31	h Y2	$G\alpha_{i/o}$	NPY	10908	7708	5387
32	h Y1	$G\alpha_{i/o}$	NPY	1879	7722	6728
33	h Y4	$G\alpha_{i/o}$	PP	9966	9422	7397
34	h α1A adr	$G\alpha_{q}$	NE	14167	9816	6597
35	h D1	$G\alpha_{s}$, $G\alpha_{i/o}$	DA	0	12120	13099
36	r NTR1	$G\alpha_{q}$	NT	11171	14476	6111

$G\alpha_{q/s}$ Chimeras

To identify additional uses for an invertebrate-based $G\alpha_{q}$ construct, modifications were made to the backbone and C-terminus of another type of chimera, $G\alpha_{q/s}$. Initially, the function of $hG\alpha_{q/s5}$ was compared with that of $hG\alpha_{q/s9}$. In one example, either construct was co-transfected into COS-7 cells with the human D1 receptor, which is typically $G\alpha_{s}$- or $G\alpha_{i/o}$-coupling (Sidhu et al., 1991). Transfected cells were stimulated with dopamine at concentrations up to 100 μM and monitored for calcium mobilization. The average maximal agonist-induced response ranged from undetectable with $hG\alpha_{q/s5}$ (n = 2) to 5692 relative fluorescent units with $hG\alpha_{q/s9}$ (n = 4). The positive effect of increasing the $G\alpha_{s}$ tail length contrasts with data for *C. elegans* $G\alpha_{q/z}$-type chimeras and has not been described previously (Conklin et

al., 1993, 1996). To further enhance signal detection, the human $G\alpha_q$ backbone was replaced with the corresponding sequence from *C. elegans* $G\alpha_q$. The modified construct, $cG\alpha_{q/s9}$, was co-transfected into COS-7 cells together with the human D1 receptor, and transfected cells were stimulated with dopamine at concentrations up to 100 μM. The average maximal dopamine-stimulated fluorescent signal with $cG\alpha_{q/s9}$ was 8692 fluorescent units (n = 4), a 1.5-fold increase over the response with $hG\alpha_{q/s9}$. To test the general utility of $cG\alpha_{q/s9}$ for detection of $G\alpha_s$-coupling receptors, this construct was co-transfected into COS-7 with a panel of 7 such GPCR. When cells were stimulated with appropriate agonists, 6/7 = 81% of the $G\alpha_s$-coupling receptors generated positive responses (> 500 fluorescence units). Further extension of the C-terminal $G\alpha_s$ tail to 21 amino acids ($cG\alpha_{q/s21}$) yielded similar results overall, both in terms of detection rate and maximal response (Table 6).

TABLE 6. $G\alpha_{q/s}$ chimeras and Gs-coupled receptors in COS-7 cells: maximum agonist response. Transfected cells were monitored for calcium mobilization in the FLIPR™ using the calcium sensitive dye Fluo-3. Maximal agonist concentration was 100 μM for non-peptide ligands or 10 μM for GLP-1 (7-36) amide. Fluorescence data represent the mean from 2 or more experiments. h = human, adr = adrenergic, DA = dopamine; GLP-1 = glucagon-like peptide; 5HT = serotonin; NE = norepinephrine

#	RECEPTOR	AGONIST	MAXIMAL SIGNAL (FLUORESCENCE UNITS)			
			$hG\alpha_{q/s5}$	$HG\alpha_{q/s9}$	$cG\alpha_{q/s9}$	$cG\alpha_{q/s21}$
1	h GLP-1	GLP-1	189	4198	2461	3120
2	h 5HT7	5HT	0	0	387	206
3	h D5	DA	0	745	1870	3385
4	h 5HT4	5HT	1709	2309	1701	1731
5	h 5HT6	5HT	98	999	1639	1009
6	h β2 adr	NE	43	1439	3106	3513
7	h D1	DA	0	5692	8692	9433

That the *C. elegans* backbone provides a signaling advantage when incorporated into either $G\alpha_{q/z}$-type or $G\alpha_{q/s}$-type chimeras suggests a novel and general method for designing effective chimeric constructs. In yet another example, human $G\alpha_{q/i3(5)}$ was compared with *C. elegans* $G\alpha_{q/i3(5)}$ using COS-7 cells transfected with the rat GALR3 receptor. The maximum signal produced by porcine galanin was 2084 relative fluorescent units with human $G\alpha_{q/i3(5)}$ (n = 4), compared to 2564 fluorescent units with *C. elegans* $G\alpha_{q/i3(5)}$ (n = 4). These data extend the range of possible uses for a *C. elegans* backbone in a $G\alpha_q$ chimeric construct.

Multiple chimerae strategies

Application of this technology to a high throughput screening paradigm (such as orphan receptor screening or expression cloning) requires that a maximal number of chimera-*dependent* receptors ($G\alpha_{i/o}$ and $G\alpha_s$-coupling) can function under the same conditions as chimera-*independent* receptors ($G\alpha_q$-coupled). One strategy, described above, is to use a single extremely promiscuous construct such as $cG\alpha_{q/z5}$.

Another strategy is to combine multiple chimeras in a transfection mixture. Ideally, the mixture should be reduced to its essential components, both in terms of individual chimera and corresponding cDNA or mRNA. A reductionist approach has several advantages: 1) it increases the allowance for cDNA or mRNA encoding the GPCR of interest; 2) it reduces potential competition for protein translation; and 3) it reduces the risk for dominant negative suppression of Gq-coupled receptor function. A simple and effective combination could be formed with a $cG\alpha_{q/z}$-type chimera and a $cG\alpha_{q/s}$-type chimera. In one example, a transfection mixture containing 2 μg $cG\alpha_{q/z9}$ cDNA, 2 μg $cG\alpha_{q/s9}$ cDNA, and 16 μg GPCR cDNA was transfected into COS-7 cells for subsequent monitoring of calcium mobilization. Out of 36 receptors tested, 28 receptors = 78% were detectable upon agonist stimulation with maximal signals > 500 fluorescence units (Table 7). The detection rate was identical to that obtained previously with $cG\alpha_{q/z5}$ or $cG\alpha_{q/z9}$ alone, except that the two chimerae together favored detection of the $G\alpha_s$-coupling receptor, human GLP-1. The use of multiple chimerae therefore represents an alternative method for screening various receptor types ($G\alpha_{i/Go}$-, $G\alpha_s$, and $G\alpha_q$-coupled) in a single assay format.

TABLE 7. Chimerae $cG\alpha_{q/z9}$ and $cG\alpha_{q/s9}$ and GPCRs in COS-7 cells: agonist-induced responses. Two μg $cG\alpha_{q/z9}$, 2 μg $cG\alpha_{q/s9}$, and 16 μg GPCR cDNA were transfected into COS-7 cells. Transfected cells were monitored for calcium mobilization in the FLIPR™ using the calcium sensitive dye Fluo-3. Maximum agonist concentrations were 100 μM for non-peptide ligands or 10 μM for peptide ligands, except for neurotensin (1 μM).

Fluorescence data represent the mean from 2 or more experiments. h = human; m = mouse; r = rat; adr = adrenergic; DA = dopamine; GLP-1 = glucagon-like peptide; 5HT = serotonin; GAL = galanin; NE = norepinephrine; MCH = melanin-concentrating hormone; NPY = neuropeptide Y; PP = pancreatic polypeptide; NPFF = neuropeptide FF; and NT = neurotensin

#	RECEPTOR	PROPOSED COUPLING	AGONIST	MAXIMAL SIGNAL (FLUORESCENCE UNITS)
				$cG\alpha_{q/z9}$ + $cG\alpha_{q/s9}$
1	h D3	$G\alpha_{i/o}$	DA	208
2	h GLP-1	$G\alpha_{s}$	GLP-1	794
3	h 5HT7	$G\alpha_{s}$	5HT	292
4	h 5HT1E	$G\alpha_{i/o}$	5HT	2
5	h 5HT1F	$G\alpha_{i/o}$	5HT	247
6	m 5HT5B	$G\alpha_{i/o}$	5HT	0
7	m 5HT5A	$G\alpha_{i/o}$	5HT	45
8	h 5HT1D	$G\alpha_{i/o}$	5HT	433
9	h D5	$G\alpha_{s}$, $G\alpha_{i/o}$	DA	1172
10	h 5HT1B	$G\alpha_{i/o}$	5HT	190
11	h 5HT4	$G\alpha_{s}$, $G\alpha_{q}$	5HT	2345
12	h 5HT6	$G\alpha_{s}$	5HT	1598
13	h GALR3	$G\alpha_{i/o}$	GAL	853
14	h β2 adr	$G\alpha_{s}$, $G\alpha_{i/o}$	NE	2346
15	h 5HT1A	$G\alpha_{i/o}$	5HT	2161
16	r GALR3	$G\alpha_{i/o}$	GAL	1402
17	h MCH	$G\alpha_{q}$	MCH	4808
18	r GALR1	$G\alpha_{i/o}$	GAL	1544
19	r Y4	$G\alpha_{i/o}$	PP	1015
20	h α2C adr	$G\alpha_{i/o}$	NE	2341

#	RECEPTOR	PROPOSED COUPLING	AGONIST	MAXIMAL SIGNAL (FLUORESCENCE UNITS)
				$cG\alpha_{q/z9}$ + $cG\alpha_{q/s9}$
21	r GALR2	$G\alpha_q$	GAL	2665
22	h α2B adr	$G\alpha_{i/o}$	NE	4855
23	h Y5	$G\alpha_{i/o}$	NPY	982
24	h GALR2	$G\alpha_q$	GAL	4630
25	h kappa	$G\alpha_{i/o}$	U-69593	3529
26	h NPFF1	$G\alpha_{i/o}$	NPFF	793
27	h NPFF2	$G\alpha_{i/o}$	NPFF	1582
28	h α2A adr	$G\alpha_{i/o}$	NE	5284
29	h D2	$G\alpha_{i/o}$	DA	5549
30	h GALR1	$G\alpha_{i/o}$	GAL	8097
31	h Y2	$G\alpha_{i/o}$	NPY	3329
32	h Y1	$G\alpha_{i/o}$	NPY	2333
33	h Y4	$G\alpha_{i/o}$	PP	4133
34	h α1A adr	$G\alpha_q$	NE	7585
35	h D1	$G\alpha_s$, $G\alpha_{i/o}$	DA	13516
36	r NTR1	$G\alpha_q$	NT	4264

Summary of the results

This work describes a functional assay with which various types and large numbers of GPCRs can be detected. The method is based on the premise that Gα proteins are derived from a common ancestor, and that the further a Gα protein is evolutionarily from the ancestral sequence, the more likely it is to contain motifs which restrict interactions to a subset of GPCRs. Conversely, sequences from more primitive organisms such as invertebrates may lack the restrictive motifs. Focusing specifically on $G\alpha_q$, we

performed an amino acid sequence alignment of all known protein structures and identified distinct motifs, which differentiate vertebrate from invertebrate species (Figure 5). For example, invertebrates lack the 6 amino acid N-terminal extension proposed to restrict GPCR interaction (Kostenis et al., 1998), and also contain Glu^{18}-Lys^{19} instead of the vertebrate Ala^{18}-Arg^{19} in a region of $G\alpha_q$ associated with receptor recognition (Lambright et al., 1996). These structural differences led us to speculate that an invertebrate $G\alpha_q$ backbone might function differently in a $G\alpha_q$ chimeric construct than would a vertebrate homologue, and that this difference might be expressed as an increase in GPCR/chimera promiscuity.

This hypothesis was tested using the invertebrate *C. elegans* as the source of the $G\alpha_q$ backbone, combined with C-terminal mammalian $G\alpha$ tails 5, 9 or 21 amino acids in length. $cG\alpha_{q/z5}$ was more promiscuous than any previously described $G\alpha$ construct, supporting receptor activation when co-transfected into *Xenopus* oocytes or mammalian COS-7 cells with most $G\alpha_{i/o}$-, $G\alpha_s$, and $G\alpha_q$-coupling receptors. This result was unexpected, and contrasts with the prevailing expectations of experts in the field (Milligan and Rees, 1999). Indeed, the current data (Conklin et al., 1993, 1996; Milligan and Rees, 1999) support the idea that each G protein chimera is only capable of functional interaction with a limited range of receptors. If true, this perceived limitation would necessitate the assay of each GPCR against a panel of chimeric G proteins in order to identify an effective GPCR/G protein combination. The results indicate that certain chimeras, such as $cG\alpha_{q/z5}$, are able to

effectively couple to a very wide number of GPCRs, thus eliminating the need for such multiple assays.

C. elegans $G\alpha_{q/z5}$ may be used alone or combined with a second chimera such as $cG\alpha_{q/s9}$ to further increase the detection rate especially for $G\alpha_s$-coupled receptors.

Conserved motifs within invertebrate $G\alpha_q$ subunits predict enhanced promiscuity from the use of any invertebrate $G\alpha_q$ backbone, including, but not limited to, the known $G\alpha_q$ sequences listed in Table 8. To provide experimental evidence for this we cloned and expressed a *D. melanogaster* chimera ($dG\alpha_{q/z5}$; Figure 2) containing the five C-terminal amino acids of human $G\alpha_z$. A comparison of $cG\alpha_{q/z5}$, $dG\alpha_{q/z5}$ and $hG\alpha_{q/z5}$ revealed that the two invertebrate chimerae show a similar enhanced coupling to D1 receptors as compared to the corresponding human chimera (Table 9). These data strongly argue against the possibility that *C. elegans* $G\alpha_q$ is somehow unique in its ability to couple promiscuously. Rather, the *D. melanogaster* data suggest that many, if not all, invertebrate $G\alpha_q$ genes may provide a similarly enhanced utility to couple to a wide variety of GPCRs.

The general utility of employing $G\alpha$ subunits from primitive organisms may be extended to include non-$G\alpha_q$ subunits from organisms outside of the animal kingdom, including for example, members of the genus *Dictyostelium*. The G-protein α subunits of *Dictyostelium discoideum* do not readily fall into those classes defined for members of the animal kingdom (Wilkie and Yokoyama, 1994), however, individual $G\alpha$ subunits such as G alpha 2 have been

shown to directly activate the PLC pathway (Okaichi et al., 1992). Other Gα subunits of *Dictyostelium*, such as G alpha 4, may also be useful based on their homology to member of the $G\alpha_q$ family. For example, G alpha 4 exhibits a greater homology to *C. elegans* $G\alpha_q$ than does G alpha 2 (47% *vs.* 42% at the amino acid level). Therefore, it is anticipated that Gα subunits from *Dictyostelium*, with or without amino acid substitutions within the protein, may be useful for functional assays for GPCRs. Therefore, for the purposes of this invention, the term invertebrate $G\alpha_q$ G protein includes Dictyostelium G alpha 2 ($G\alpha_2$) and G alpha 4 ($G\alpha_4$) G proteins.

Further enhancements to the coupling scope of the chimeric G proteins described in this invention may be realized by making select point mutations within regions of the protein known to contact GPCRs. For example, amino acids within the alpha4 helical domain of $G\alpha_{i1}$ are important for permitting a productive coupling to the 5HT1B receptor (Bae et al., 1999). Mutations altering two amino acids in this domain, Q304 and E308, specifically prevent coupling to 5HT1B. The majority of receptors that did not couple productively to the chimeric G proteins described herein include several members of the 5HT1 subfamily, including 5HT1B. It is predicted, therefore, based on the work of Bae et al. (1999) that making homologous amino acid substitutions in the alpha4 region of $cG\alpha_q$ would extend the number of GPCRs that can functionally couple to chimeras, composed of $cG\alpha_q$.

TABLE 8. Description of $G\alpha_q$ subunits from invertebrates useful for construction of chimeras.

SPECIES	COMMON NAME	DESIGNATION	GENBANK ACCESSION NUMBER
Drosophila melanogaster	Fruit fly	GBQ1_drome	P23625
Drosophila melanogaster	Fruit fly	GBQ3_drome	P54400
Limulus polyphemus	Horseshoe crab	GBQ_limpo	g1857923
Patinopecten yessoensis	Scallop	GBQ_patye	O15975
Loligo forbesi	Squid	GBQ_lolfo	P38412
Homarus americanus	Lobster	GBQ_homam	P91950
Lymnaea stagnalis	Pond snail	GBQ_lymst	P38411
Geodia cydonium	Sponge	GBQ_geocy	Y14248
Caenorhabditis elegans	Nematode	GBQ_caeel	AF003739

TABLE 9. Comparison of invertebrate chimerae $dG\alpha_{q/z5}$ and $cG\alpha_{q/z5}$ with two different human $hG\alpha_{q/z5}$ chimerae in their ability to couple to human D1 receptors in COS-7 cells. Ten µg chimera cDNA and 10 µg of human D1 receptor cDNA were transfected into COS-7 cells. Transfected cells were monitored for calcium mobilization in the FLIPR™ using the calcium sensitive dye Fluo-3. Maximum agonist concentration was 100 µM dopamine. Fluorescent data represent the mean from two experiments.

MAXIMAL SIGNAL (FLUORESCENCE UNITS)			
$dG\alpha_{q/z5}$	$cG\alpha_{q/z5}$	$hG\alpha_{q/z5}$*	$hG\alpha_{q/z5}$†
2149	4832	0	0

* Identical to Accession number L76256.

† Ala → Ser substitution at position 171.

This invention provides a powerful and rapid system for detecting GPCR activation that is obtained when an invertebrate-based $G\alpha_q$ chimera is coupled to a signal amenable to high throughput screening, such as fluorescence-based detection of calcium mobilization. Specific applications would include: 1) high throughput screening and pharmacological analysis of a known GPCR, e.g., drug discovery; 2) screening of ligands against a cloned orphan receptor whose signaling pathways are unknown; and 3) screening of a cDNA library against one or more ligands in an expression cloning paradigm. In each case, this method supports detection of GPCRs from various classes ($G\alpha_{i/o}$, $G\alpha_s$-, and $G\alpha_q$-coupling) in a single assay format with greater efficiency and capture rate than previously described methods.

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What is claimed is:

1. An isolated nucleic acid encoding a chimeric G protein, wherein the chimeric G protein comprises an invertebrate Gαq G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate G protein beginning with the C-terminal amino acid of such vertebrate G protein, wherein such number equals the number of amino acids deleted or varies therefrom by no more than five amino acids, provided that at least five of the C-terminal amino acids of the chimeric G protein are present at the C-terminus of such vertebrate G protein.

2. A nucleic acid of claim 1, wherein the chimeric G protein comprises an invertebrate Gαq G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate G protein beginning with the C-terminal amino acid of such vertebrate G protein, wherein such number equals the number of amino acids deleted or varies therefrom by no more than two amino acids, provided that at least five of the C-terminal amino acids of the chimeric G protein are present at the C-terminus of such vertebrate G protein.

3. A nucleic acid of claim 1, wherein the chimeric G protein comprises an invertebrate Gαq G protein

from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate G protein beginning with the C-terminal amino acid of such vertebrate G protein, wherein such number equals the number of amino acids deleted.

4. The nucleic acid of claim 1, wherein the nucleic acid is DNA.

5. The nucleic acid of claim 4, wherein the DNA is cDNA.

6. The nucleic acid of claim 4, wherein the DNA is genomic DNA.

7. The nucleic acid of claim 1, wherein the nucleic acid is RNA.

8. The nucleic acid of claim 1, wherein the vertebrate G protein is a mammalian G protein.

9. The nucleic acid of claim 1, wherein the contiguous amino acids which have been deleted are contained in FVFAAVKDTILQHNLKEYNLV* (SEQ ID NO: 37), wherein V* is the C-terminal amino acid.

10. The nucleic acid of claim 1, wherein the vertebrate G protein is a vertebrate Gαz G protein.

11. The nucleic acid of claim 10, wherein the number of contiguous amino acids which have replaced the deleted amino acids are contained in

FVFDAVTDVIIQNNLKYIGLC* (SEQ ID NO: 38), wherein C* is the C-terminal amino acid.

12. The nucleic acid of claim 10, wherein the invertebrate Gαq G protein has five contiguous amino acids beginning with the C-terminal amino acid which have been deleted and replaced by five contiguous amino acids beginning with the C-terminal amino acid of the vertebrate Gαz protein.

13. The nucleic acid of claim 1, wherein the vertebrate G protein is a vertebrate Gαs G protein.

14. The nucleic acid of claim 13, wherein the number of contiguous amino acids which have replaced the deleted amino acids are contained in RVFNDCRDIIQRMHLRQYELL* (SEQ ID NO: 39), wherein L* is the C-terminal amino acid.

15. The nucleic acid of claim 13, wherein the invertebrate Gαq G protein has nine contiguous amino acids beginning with the C-terminal amino acid which have been deleted and replaced by nine contiguous amino acids beginning with the C-terminal amino acid of the vertebrate Gαs protein.

16. The nucleic acid of claim 1, wherein the vertebrate G protein is a vertebrate Gαi3 G protein.

17. The nucleic acid of claim 16, wherein the number of contiguous amino acids which have replaced the

deleted amino acids are contained in FVFDAVTDVIIKNNLKECGLY* (SEQ ID NO: 40), wherein Y* is the C-terminal amino acid.

18. The nucleic acid of claim 16, wherein the invertebrate Gαq G protein has five contiguous amino acids beginning with the C-terminal amino acid which have been deleted and replaced by five contiguous amino acids beginning with the C-terminal amino acid of the vertebrate Gαi3 protein.

19. The nucleic acid of claim 1, wherein the vertebrate G protein is a vertebrate Gαi1 G protein, a vertebrate Gαi2 G protein, a vertebrate GαoA G protein, or a vertebrate GαoB G protein.

20. The nucleic acid of claim 1, wherein the invertebrate Gαq G protein is a *Caenorhabditis elegans* Gαq G protein.

21. The nucleic acid of claim 1, wherein the invertebrate Gαq G protein is a *Drosophila melanogaster* Gαq G protein, a *Limulus polyphemus* Gαq G protein, a *Patinopecten yessoensis* Gαq G protein, a *Loligo forbesi* Gαq G protein, a *Homarus americanus* Gαq G protein, a *Lymnaea stagnalis* Gαq G protein, a *Geodia cydonium* Gαq G protein, or a *Dictyostelium discoideum* $G\alpha_4$ G protein.

22. The nucleic acid of claim 1, wherein the chimeric G protein has an amino acid sequence substantially the same as the amino acid sequence shown in (a) Figure 2, *C. elegans* $G\alpha_{q/z5}$ (SEQ ID NO: 1); (b) Figure 2, *C. elegans* $G\alpha_{q/z9}$ (SEQ ID NO: 2); (c) Figure 2, *C. elegans* $G\alpha_{q/s9}$ (SEQ ID NO: 3); (d) Figure 2, *C. elegans* $G\alpha_{q/s21}$ (SEQ ID NO: 4); (e) Figure 2, *C. elegans* $G\alpha_{q/i3(5)}$ (SEQ ID NO: 5); or (f) Figure 2, *D. melaongaster* $G\alpha_{q/zs}$ (SEQ ID NO: 41).

23. A vector comprising the nucleic acid of claim 1.

24. A vector of claim 23 adapted for expression in a cell which comprises the regulatory elements necessary for expression of the nucleic acid in the cell operatively linked to the nucleic acid encoding the chimeric G protein so as to permit expression thereof, wherein the cell is a bacterial, amphibian, yeast, insect, or mammalian cell.

25. The vector of claim 24, wherein the vector is a plasmid, a baculovirus, or a retrovirus.

26. A cell comprising the vector of claim 23, wherein the cell comprises DNA encoding a mammalian G protein-coupled receptor.

27. A cell of claim 26, wherein the DNA encoding the mammalian G protein-coupled receptor is endogenous to the cell.

28. A cell of claim 26, wherein the DNA encoding the mammalian G protein-coupled receptor is transfected into the cell.

29. A cell of claim 26, wherein the cell is a non-mammalian cell.

30. A cell of claim 29, wherein the non-mammalian cell is a *Xenopus* oocyte cell or a *Xenopus* melanophore cell.

31. A cell of claim 26, wherein the cell is a mammalian cell.

32. A mammalian cell of claim 31, wherein the cell is a COS-7 cell, a 293 human embryonic kidney cell, a NIH-3T3 cell, a LM(tk-) cell, a mouse Y1 cell, or a CHO cell.

33. A cell of claim 26, wherein the cell is an insect cell.

34. An insect cell of claim 33, wherein the insect cell is an Sf9 cell, an Sf21 cell or a Trichoplusia *ni* 5B-4 cell.

35. A membrane preparation isolated from the cell of any one of claims 26, 27, 28, 29, 31, 32, 33 or 34.

36. A process for determining whether a chemical compound is a mammalian G protein-coupled receptor agonist which comprises contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor, with the

compound under conditions permitting the activation of the mammalian G protein-coupled receptor, and detecting an increase in mammalian G protein-coupled receptor activity, so as to thereby determine whether the compound is a mammalian G protein-coupled receptor agonist.

37. A process for determining whether a chemical compound is a mammalian G protein-coupled receptor agonist which comprises contacting a membrane preparation from cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor, with the compound under conditions permitting the activation of the mammalian G protein-coupled receptor, and detecting an increase in mammalian G protein-coupled receptor activity, so as to thereby determine whether the compound is a mammalian G protein-coupled receptor agonist.

38. A process for determining whether a chemical compound is a mammalian G protein-coupled receptor antagonist which comprises contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor, with the compound in the presence of a known mammalian G protein-coupled receptor agonist, under conditions permitting the activation of the mammalian G protein-coupled receptor, and detecting a decrease in mammalian G protein-coupled receptor activity, so as to thereby determine whether the compound is a mammalian G protein-coupled receptor antagonist.

39. A process for determining whether a chemical compound is a mammalian G protein-coupled receptor antagonist which comprises contacting a membrane preparation from cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor, with the compound in the presence of a known mammalian G protein-coupled receptor agonist, under conditions permitting the activation of the mammalian G protein-coupled receptor, and detecting a decrease in mammalian G protein-coupled receptor activity, so as to thereby determine whether the compound is a mammalian G protein-coupled receptor antagonist.

40. The process of claim 36, 37, 38, or 39, wherein the DNA encoding the mammalian G protein-coupled receptor is endogenous to the cell.

41. The process of claim 36, 37, 38, or 39, wherein the DNA encoding the mammalian G protein-coupled receptor is transfected into the cell.

42. The process of claim 36, 37, 38, or 39, wherein the mammalian G protein-coupled receptor is a human Y5 receptor, a human GALR2 receptor, a human kappa opioid receptor, a human NPFF1 receptor, a human NPFF2 receptor, a human α2A adrenergic receptor, a human dopamine D2 receptor, a human GALR1 receptor, a human Y2 receptor, a human Y1 receptor, a human Y4 receptor, a human α1A adrenergic receptor, a human dopamine D1 receptor, or a rat NTR1 receptor.

43. A process for determining whether a chemical compound specifically binds to and activates a mammalian G protein-coupled receptor, which comprises contacting cells producing a second messenger response, expressing the DNA encoding the mammalian G protein-coupled receptor, and expressing the DNA encoding a chimeric G protein, wherein such cells do not normally express the DNA encoding the chimeric G protein, with the chemical compound under conditions suitable for activation of the mammalian G protein-coupled receptor, and measuring the second messenger response in the presence and in the absence of the chemical compound, a change in the second messenger response in the presence of the chemical compound indicating that the compound activates the mammalian G protein-coupled receptor.

44. The process of claim 43, wherein the DNA encoding the mammalian G protein-coupled receptor is endogenous to the cell.

45. The process of claim 43, wherein the DNA encoding the mammalian G protein-coupled receptor is transfected into the cell.

46. The process of claim 43, wherein the second messenger response is the detection of a reporter protein under the transcriptional control of a promoter element.

47. The process of claim 43, wherein the second messenger response is measured by a change in cell proliferation.

48. The process of claim 43, wherein the second messenger response is a Gαq second messenger response.

49. The process of claim 48, wherein the Gαq second messenger response comprises release of inositol phosphate and the change in second messenger is an increase in the level of inositol phosphate.

50. The process of claim 48, wherein the Gαq second messenger response comprises release of arachidonic acid and the change in second messenger is an increase in the level of arachidonic acid.

51. The process of claim 48, wherein the Gαq second messenger response comprises activation of MAP kinase and the change in second messenger response is an increase in MAP kinase activation.

52. The process of claim 48, wherein the Gαq second messenger response comprises intracellular calcium levels and the change in second messenger is an increase in the measure of intracellular calcium.

53. The process of claim 52, wherein the measure of intracellular calcium levels is made by chloride current readings.

54. The process of claim 52, wherein the measure of intracellular calcium is made by fluorescence readings, luminescence readings, electrophysiological readings, or through the

detection of a reporter protein under the transcriptional control of a calcium-responsive promoter element.

55. A process for determining whether a chemical compound specifically binds to and inhibits activation of a mammalian G protein-coupled receptor, which comprises separately contacting cells producing a second messenger response, expressing the DNA encoding the mammalian G protein-coupled receptor, and expressing the DNA encoding a chimeric G protein, wherein such cells do not normally express the DNA encoding the chimeric G protein, with both the chemical compound and a second chemical compound known to activate the mammalian G protein-coupled receptor, and with only the second chemical compound, under conditions suitable for activation of the mammalian G protein-coupled receptor, and measuring the second messenger response in the presence of only the second chemical compound and in the presence of both the second chemical compound and the chemical compound, a smaller change in the second messenger response in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound indicating that the chemical compound inhibits activation of the mammalian G protein-coupled receptor.

56. The process of claim 55, wherein the DNA encoding the mammalian G protein-coupled receptor is endogenous to the cell.

57. The process of claim 55, wherein the DNA encoding the mammalian G protein-coupled receptor is transfected into the cell.

58. The process of claim 55, wherein the second messenger response is the detection of a reporter protein under the transcriptional control of a promoter element.

59. The process of claim 55, wherein the second messenger response is measured by a change in cell proliferation.

60. The process of claim 55, wherein the second messenger response is a Gαq second messenger response.

61. The process of claim 60, wherein the Gαq second messenger response comprises release of inositol phosphate and the change in second messenger response is a smaller increase in the level of inositol phosphate in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound.

62. The process of claim 60, wherein the Gαq second messenger response comprises activation of MAP kinase and the change in second messenger response is a smaller increase in the level of MAP kinase activation in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound.

63. The process of claim 60, wherein the Gαq second messenger response comprises release of arachidonic acid and the change in second messenger response is an increase in the level of arachidonic acid levels in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound.

64. The process of claim 60, wherein the Gαq second messenger response comprises change in intracellular calcium levels and the change in second messenger response is a smaller increase in the measure of intracellular calcium in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound.

65. The process of claim 64, wherein the measure of intracellular calcium levels is made by chloride current readings.

66. The process of claim 64, wherein the measure of intracellular calcium is made by fluorescence readings, luminescence readings, electrophysiological readings, or through the detection of a reporter protein under the transcriptional control of a calcium-responsive promoter element.

67. A process of screening a plurality of chemical compounds not known to activate a mammalian G protein-coupled receptor to identify a compound which activates the mammalian G protein-coupled receptor which comprises:

(a) contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor with the plurality of compounds not known to activate the mammalian G protein-coupled receptor, under conditions permitting activation of the mammalian G protein-coupled receptor;

(b) determining whether the activity of the mammalian G protein-coupled receptor is increased in the presence of one or more of the compounds; and if so

(c) separately determining whether the activation of the mammalian G protein-coupled receptor is increased by any compound included in the plurality of compounds, so as to thereby identify each compound which activates the mammalian G protein-coupled receptor.

68. A process of screening a plurality of chemical compounds not known to inhibit the activation of a mammalian G protein-coupled receptor to identify a compound which inhibits the activation of the mammalian G protein-coupled receptor, which comprises:

(a) contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor with the plurality of compounds in the presence of a known mammalian G protein-coupled

receptor agonist, under conditions permitting activation of the mammalian G protein-coupled receptor;

(b) determining whether the extent or amount of activation of the mammalian G protein-coupled receptor is reduced in the presence of one or more of the compounds, relative to the extent or amount of activation of the mammalian G protein-coupled receptor in the absence of such one or more compounds; and if so

(c) separately determining whether each such compound inhibits activation of the mammalian G protein-coupled receptor for each compound included in the plurality of compounds, so as to thereby identify any compound included in such plurality of compounds which inhibits the activation of the mammalian G protein-coupled receptor.

69. The process of claim 67 or 68, wherein the DNA encoding the mammalian G protein-coupled receptor is endogenous to the cell.

70. The process of claim 67 or 68, wherein the DNA encoding the mammalian G protein-coupled receptor is transfected into the cell.

71. A process for determining whether a chemical compound is a mammalian G protein-coupled receptor agonist, which comprises separately contacting membrane preparations from cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a

mammalian G protein-coupled receptor with both the compound and [^{35}S]GTPγS, and with only [^{35}S]GTPγS, under conditions permitting the activation of the mammalian G protein-coupled receptor, and detecting [^{35}S]GTPγS binding to the membrane preparation and an increase in [^{35}S]GTPγS binding in the presence of the compound indicating that the chemical compound activates the mammalian G protein-coupled receptor.

72. A process for determining whether a chemical compound is a mammalian G protein-coupled receptor antagonist which comprises separately contacting membrane preparations from cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor with the chemical compound, [^{35}S]GTPγS, and a second chemical compound known to activate the mammalian G protein-coupled receptor, with [^{35}S]GTPγS and only the second compound, and with [^{35}S]GTPγS alone, under conditions permitting the activation of the mammalian G protein-coupled receptor, detecting [^{35}S]GTPγS binding to each membrane preparation, comparing the increase in [^{35}S]GTPγS binding in the presence of the compound and the second compound relative to the binding of [^{35}S]GTPγS alone to the increase in [^{35}S]GTPγS binding in the presence of the second chemical compound relative to the binding of [^{35}S]GTPγS alone, and detecting a smaller increase in [^{35}S]GTPγS binding in the presence of the compound and the second compound indicating that the compound is a mammalian G protein-coupled receptor antagonist.

73. The process of claim 71 or 72, wherein the DNA encoding the mammalian G protein-coupled receptor is endogenous to the cell.

74. The process of claim 71 or 72, wherein the DNA encoding the mammalian G protein-coupled receptor is transfected into the cell.

75. The process of claim 71 or 72, wherein the mammalian G protein-coupled receptor produces a Gαs second messenger response in the absence of the chimeric G protein.

76. A process for determining whether a chemical compound is a mammalian G protein-coupled receptor agonist, which comprises contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor with a compound, under conditions permitting the activation of the mammalian G protein-coupled receptor, and detecting changes in receptor active state conformation as manifested by changes in receptor/G protein heterotrimer association/dissociation in the presence of the compound indicating that the chemical compound activates the mammalian G protein-coupled receptor.

77. A process for determining whether a chemical compound is a mammalian G protein-coupled receptor antagonist which comprises separately contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled

receptor with the chemical compound in the presence of a known mammalian G protein-coupled receptor agonist, under conditions permitting the activation of the mammalian G protein-coupled receptor, and detecting changes in receptor active state conformation as manifested by changes in receptor/G protein heterotrimer association/dissociation in the presence of the compound indicating that the compound is a mammalian G protein-coupled receptor antagonist.

78. The process of claim 76 or 77, wherein the DNA encoding the mammalian G protein-coupled receptor is endogenous to the cell.

79. The process of claim 76 or 77, wherein the DNA encoding the mammalian G protein-coupled receptor is transfected into the cell.

80. The process of any one of claims 36, 37, 38, 39, 43, 55, 67, 68, 71, 72, 76, or 77, wherein the chimeric G protein comprises an invertebrate Gαq G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate G protein beginning with the C-terminal amino acid of such vertebrate G protein, wherein such number equals the number of amino acids deleted.

81. The process of any one of claims 36, 37, 38, 39, 43, 55, 67, 68, 71, 72, 76, or 77, wherein the chimeric G protein comprises an invertebrate Gαq G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning

with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate Gαz protein beginning with the C-terminal amino acid of such vertebrate Gαz protein, wherein such number equals the number of amino acids deleted.

82. The process of any one of claims 36, 37, 38, 39, 43, 55, 67, 68, 71, 72, 76, or 77, wherein the chimeric G protein comprises an invertebrate Gαq G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate Gαs protein beginning with the C-terminal amino acid of such vertebrate Gαs protein, wherein such number equals the number of amino acids deleted.

83. The process of any one of claims 36, 37, 38, 39, 43, 55, 67, 68, 71, 72, 76, or 77, wherein the chimeric G protein comprises an invertebrate Gαq G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate Gαi3 protein beginning with the C-terminal amino acid of such vertebrate Gαi3 protein, wherein such number equals the number of amino acids deleted.

84. The process of any one of claims 36, 37, 38, 39, 43, 55, 67, 68, 71, 72, 76, or 77, wherein the chimeric G protein comprises a *Caenorhabditis elegans* Gαq G protein from which at least five,

but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate G protein beginning with the C-terminal amino acid of such vertebrate G protein, wherein such number equals the number of amino acids deleted.

85. The process of any one of claims 36, 37, 38, 39, 43, 55, 67, 68, 71, 72, 76, or 77, wherein the chimeric G protein comprises a *Drosophila melanogaster* Gαq G protein, a *Limulus polyphemus* Gαq G protein, a *Patinopecten yessoensis* Gαq G protein, a *Loligo forbesi* Gαq G protein, a *Homarus americanus* Gαq G protein, a *Lymnaea stagnalis* Gαq G protein, a *Geodia cydonium* Gαq G protein, or a *Dictyostelium discoideum* $G\alpha_4$ G protein, from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate G protein beginning with the C-terminal amino acid of a vertebrate G protein, wherein such number equals the number of amino acids deleted.

86. The process of any one of claims 36, 37, 38, 39, 43, 55, 67, 68, 71, 72, 76, or 77, wherein the chimeric G protein has an amino acid sequence substantially the same as the amino acid sequence shown in (a) Figure 2, *C. elegans* $G\alpha_{q/z5}$ (SEQ ID NO: 1); (b) Figure 2, *C. elegans* $G\alpha_{q/z9}$ (SEQ ID NO: 2); (c) Figure 2, *C. elegans* $G\alpha_{q/s9}$ (SEQ ID NO: 3); (d) Figure 2, *C. elegans* $G\alpha_{q/s21}$ (SEQ ID NO: 4); (e) Figure 2, *C. elegans* $G\alpha_{q/i3(5)}$ (SEQ ID

NO: 5); or (f) Figure 2, *D. melaongaster* $G\alpha_{q/zs}$ (SEQ ID NO: 41).

87. The process of any one of claims 36, 37, 38, 39, 43, 55, 67, 68, 71, 72, 76, or 77, wherein the cell is an insect cell.

88. The process of any one of claims 36, 37, 38, 39, 43, 55, 67, 68, 71, 72, 76, or 77, wherein the cell is a mammalian cell.

89. The process of claim 88, wherein the cell is nonneuronal in origin.

90. The process of claim 89, wherein the nonneuronal cell is a COS-7 cell, 293 human embryonic kidney cell, a CHO cell, a NIH-3T3 cell, a mouse Y1 cell, or a LM(tk-) cell.

91. A process for identifying a chemical compound which specifically binds to a mammalian G protein-coupled receptor which comprises contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor, wherein such cells do not normally express the DNA encoding the chimeric G protein, with the compound under conditions suitable for binding, and detecting specific binding of the chemical compound to the mammalian G protein-coupled receptor.

92. A process for identifying a chemical compound which specifically binds to a mammalian G protein-coupled receptor which comprises contacting a membrane preparation from cells

transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor, wherein such cells do not normally express the DNA encoding the chimeric G protein, with the compound under conditions suitable for binding, and detecting specific binding of the chemical compound to the mammalian G protein-coupled receptor.

93. A process involving competitive binding for identifying a chemical compound which specifically binds to a mammalian G protein-coupled receptor which comprises separately contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor, wherein such cells do not normally express the DNA encoding the chimeric G protein, with both the chemical compound and a second chemical compound known to bind to the mammalian G protein-coupled receptor, and with only the second chemical compound, under conditions suitable for binding of both compounds, and detecting specific binding of the chemical compound to the mammalian G protein-coupled receptor, a decrease in the binding of the second chemical compound to the mammalian G protein-coupled receptor in the presence of the chemical compound indicating that the chemical compound binds to the mammalian G protein-coupled receptor.

94. A process involving competitive binding for identifying a chemical compound which specifically binds to a mammalian G protein-

coupled receptor which comprises separately contacting a membrane preparation from cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor, wherein such cells do not normally express the DNA encoding the chimeric G protein, with both the chemical compound and a second chemical compound known to bind to the receptor, and with only the second chemical compound, under conditions suitable for binding of both compounds, and detecting specific binding of the chemical compound to the mammalian G protein-coupled receptor, a decrease in the binding of the second chemical compound to the mammalian G protein-coupled receptor in the presence of the chemical compound indicating that the chemical compound binds to the mammalian G protein-coupled receptor.

95. A process of screening a plurality of chemical compounds not known to bind to a mammalian G protein-coupled receptor to identify a compound which specifically binds to the mammalian G protein-coupled receptor, which comprises

(a) contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor with a compound known to bind specifically to the mammalian G protein-coupled receptor;

(b) contacting the cells of step (a) with the plurality of compounds not known to

bind specifically to the mammalian G protein-coupled receptor, under conditions permitting binding of compounds known to bind to the mammalian G protein-coupled receptor;

(c) determining whether the binding of the compound known to bind to the mammalian G protein-coupled receptor is reduced in the presence of the plurality of compounds, relative to the binding of the compound in the absence of the plurality of compounds; and if so

(d) separately determining the binding to the mammalian G protein-coupled receptor of each compound included in the plurality of compounds, so as to thereby identify any compound included therein which specifically binds to the mammalian G protein-coupled receptor.

96. A process of screening a plurality of chemical compounds not known to bind to a mammalian G protein-coupled receptor to identify a compound which specifically binds to the mammalian G protein-coupled receptor, which comprises

(a) contacting a membrane preparation from cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor with the plurality of compounds not known to bind specifically to the mammalian G protein-coupled receptor under conditions

permitting binding of compounds known to bind to the mammalian G protein-coupled receptor;

(b) determining whether the binding of a compound known to bind to the mammalian G protein-coupled receptor is reduced in the presence of the plurality of compounds, relative to the binding of the compound in the absence of the plurality of compounds; and if so

(c) separately determining the binding to the mammalian G protein-coupled receptor of each compound included in the plurality of compounds, so as to thereby identify any compound included therein which specifically binds to the mammalian G protein-coupled receptor.

97. The process of claim 91, 92, 93, 94, 95, or 96, wherein the DNA encoding the mammalian G protein-coupled receptor is endogenous to the cell.

98. The process of claim 91, 92, 93, 94, 95, or 96, wherein the DNA encoding the mammalian G protein-coupled receptor is transfected into the cell.

99. The process of any one of claims 91, 92, 93, 94, 95, or 96, wherein the chimeric G protein comprises an invertebrate Gαq G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate G protein beginning with

the C-terminal amino acid of such vertebrate G protein, wherein such number equals the number of amino acids deleted.

100. The process of any one of claims 91, 92, 93, 94, 95, or 96, wherein the chimeric G protein comprises an invertebrate Gαq G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate Gαz protein beginning with the C-terminal amino acid of such vertebrate Gαz protein, wherein such number equals the number of amino acids deleted.

101. The process of any one of claims 91, 92, 93, 94, 95, or 96, wherein the chimeric G protein comprises an invertebrate Gαq G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate Gαs protein beginning with the C-terminal amino acid of such vertebrate Gαs protein, wherein such number equals the number of amino acids deleted.

102. The process of any one of claims 91, 92, 93, 94, 95, or 96, wherein the chimeric G protein comprises an invertebrate Gαq G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids

present in a vertebrate Gαi3 protein beginning with the C-terminal amino acid of such vertebrate Gαi3 protein, wherein such number equals the number of amino acids deleted.

103. The process of any one of claims 91, 92, 93, 94, 95, or 96, wherein the chimeric G protein comprises an *Caenorhabditis elegans* Gαq G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate G protein beginning with the C-terminal amino acid of such vertebrate G protein, wherein such number equals the number of amino acids deleted.

104. The process of any one of claims 91, 92, 93, 94, 95, or 96, wherein the chimeric G protein comprises a *Drosophila melanogaster* Gαq G protein, a *Limulus polyphemus* Gαq G protein, a *Patinopecten yessoensis* Gαq G protein, a *Loligo forbesi* Gαq G protein, a *Homarus americanus* Gαq G protein, a *Lymnaea stagnalis* Gαq G protein, a *Geodia cydonium* Gαq G protein, or a *Dictyostelium discoideum* $G\alpha_4$ G protein, from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate G protein beginning with the C-terminal amino acid of such vertebrate G protein, wherein such number equals the number of amino acids deleted.

105. The process of any one of claims 91, 92, 93, 94, 95, or 96, wherein the chimeric G protein has an amino acid sequence substantially the same as the amino acid sequence shown in (a) Figure 2, *C. elegans* $G\alpha_{q/z5}$ (SEQ ID NO: 1); (b) Figure 2, *C. elegans* $G\alpha_{q/z9}$ (SEQ ID NO: 2); (c) Figure 2, *C. elegans* $G\alpha_{q/s9}$ (SEQ ID NO: 3); (d) Figure 2, *C. elegans* $G\alpha_{q/s21}$ (SEQ ID NO: 4); (e) Figure 2, *C. elegans* $G\alpha_{q/i3(5)}$ (SEQ ID NO: 5); or (f) Figure 2, *D. melaongaster* $G\alpha_{q/zs}$ (SEQ ID NO: 41).

106. The process of any one of claims 91, 92, 93, 94, 95, or 96, wherein the cell is an insect cell.

107. The process of any one of claims 91, 92, 93, 94, 95, or 96, wherein the cell is a mammalian cell.

108. The process of claim 107, wherein the cell is nonneuronal in origin.

109. The process of claim 108, wherein the nonneuronal cell is a COS-7 cell, 293 human embryonic kidney cell, a CHO cell, a NIH-3T3 cell, a mouse Y1 cell, or a LM(tk-) cell.

110. The process for making a composition of matter which specifically binds to a mammalian G protein-coupled receptor which comprises identifying a chemical compound using the process of any of claims 36, 37, 43, 67, 71, or 76 and then synthesizing the chemical compound or a novel structural and functional analog or homolog thereof.

111. The process for making a composition of matter which specifically binds to a mammalian G protein-coupled receptor which comprises identifying a chemical compound using the process of any of claims 38, 39, 55, 68, 72, or 77 and then synthesizing the chemical compound or a novel structural and functional analog or homolog thereof.

112. The process for making a composition of matter which specifically binds to a mammalian G protein-coupled receptor which comprises identifying a chemical compound using the process of any of claims 91, 92, 93, 94, 95, or 96 and then synthesizing the chemical compound or a novel structural and functional analog or homolog thereof.

113. The process for preparing a composition which comprises admixing a carrier and a pharmaceutically effective amount of a chemical compound identified by the process of any of claims 36, 37, 43, 67, 71, or 76 or a novel structural and functional analog or homolog thereof.

114. The process for preparing a composition which comprises admixing a carrier and a pharmaceutically effective amount of a chemical compound identified by the process of any of claims 38, 39, 55, 68, 72, or 77 or a novel structural and functional analog or homolog thereof.

115. The process for preparing a composition which comprises admixing a carrier and a

pharmaceutically effective amount of a chemical compound identified by the process of any of claims 91, 92, 93, 94, 95, or 96 or a novel structural and functional analog or homolog thereof.

116. A process for determining whether a chemical compound is a ligand for a mammalian G protein-coupled receptor which comprises contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor, with the compound under conditions permitting the activation of the mammalian G protein-coupled receptor, and detecting an increase in mammalian G protein-coupled receptor activity, so as to thereby determine whether the compound activates the mammalian G protein-coupled receptor and is a ligand for the mammalian G protein-coupled receptor.

117. A process for determining whether a chemical compound is a ligand for a mammalian G protein-coupled receptor which comprises contacting a membrane preparation from cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor, with the compound under conditions permitting the activation of the mammalian G protein-coupled receptor, and detecting an increase in mammalian G protein-coupled receptor activity, so as to thereby determine whether the compound activates the mammalian G protein-coupled receptor and is a ligand for the mammalian G protein-coupled receptor.

118. A process for determining whether a chemical compound is a ligand for a mammalian G protein-coupled receptor which comprises contacting cells producing a second messenger response, expressing the DNA encoding the mammalian G protein-coupled receptor, and expressing the DNA encoding a chimeric G protein, wherein such cells do not normally express the DNA encoding the chimeric G protein, with the chemical compound under conditions suitable for activation of the mammalian G protein-coupled receptor, and measuring the second messenger response in the presence and in the absence of the chemical compound, a change in the second messenger response in the presence of the chemical compound indicating that the compound activates the mammalian G protein-coupled receptor and is a ligand for the mammalian G protein-coupled receptor.

119. The process of claim 118, wherein the second messenger response is a Gαq second messenger response.

120. The process of claim 119, wherein the Gαq second messenger response comprises intracellular calcium levels and the change in second messenger is an increase in the measure of intracellular calcium.

121. The process of claim 120, wherein the measure of intracellular calcium levels is made by chloride current readings.

122. The process of claim 120, wherein the measure of intracellular calcium is made by fluorescence readings, luminescence readings, electrophysiological readings, or through the detection of a reporter protein under the transcriptional control of a calcium-responsive promoter element.

123. A process of screening a plurality of chemical compounds not known to activate a mammalian G protein-coupled receptor to identify a ligand for the mammalian G protein-coupled receptor which comprises:

(a) contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor with the plurality of compounds not known to activate the mammalian G protein-coupled receptor, under conditions permitting activation of the mammalian G protein-coupled receptor;

(b) determining whether the activity of the mammalian G protein-coupled receptor is increased in the presence of one or more of the compounds; and if so

(c) separately determining whether the activation of the mammalian G protein-coupled receptor is increased by any compound included in the plurality of compounds, so as to thereby identify each compound which activates the mammalian G protein-coupled receptor and is a ligand

for the mammalian G protein-coupled receptor.

124. A process for determining whether a chemical compound is a ligand for a mammalian G protein-coupled receptor, which comprises separately contacting membrane preparations from cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor with both the compound and [^{35}S]GTPγS, and with only [^{35}S]GTPγS, under conditions permitting the activation of the mammalian G protein-coupled receptor, and detecting [^{35}S]GTPγS binding to the membrane preparation and an increase in [^{35}S]GTPγS binding in the presence of the compound indicating that the chemical compound activates the mammalian G protein-coupled receptor and is a ligand for the mammalian G protein-coupled receptor.

125. A process for determining whether a chemical compound is a ligand for the mammalian G protein-coupled receptor, which comprises contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor with a compound, under conditions permitting the activation of the mammalian G protein-coupled receptor, and detecting changes in receptor active state conformation as manifested by changes in receptor/G protein heterotrimer association/dissociation in the presence of the compound indicating that the chemical compound activates the mammalian G

protein-coupled receptor and is a ligand for the mammalian G protein-coupled receptor.

126. A process for identifying a ligand for a mammalian G protein-coupled receptor which comprises contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor, wherein such cells do not normally express the DNA encoding the chimeric G protein, with the compound under conditions suitable for binding, and detecting specific binding of the chemical compound to the mammalian G protein-coupled receptor, indicating that the compound is a ligand for the mammalian G protein-coupled receptor.

127. A process for identifying a chemical compound which specifically binds to a mammalian G protein-coupled receptor which comprises contacting a membrane preparation from cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a mammalian G protein-coupled receptor, wherein such cells do not normally express the DNA encoding the chimeric G protein, with the compound under conditions suitable for binding, and detecting specific binding of the chemical compound to the mammalian G protein-coupled receptor, indicating that the compound is a ligand for the mammalian G protein-coupled receptor.

128. The process of claim 116, 117, 118, 123, 124, 125, 126, or 127, wherein the DNA encoding the

mammalian G protein-coupled receptor is endogenous to the cell.

129. The process of claim 116, 117, 118, 123, 124, 125, 126, or 127, wherein the DNA encoding the mammalian G protein-coupled receptor is transfected into the cell.

130. The process of any one claims 116, 117, 118, 123, 124, 125, 126, or 127, wherein the chimeric G protein comprises an invertebrate Gαq G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate G protein beginning with the C-terminal amino acid of such vertebrate G protein, wherein such number equals the number of amino acids deleted.

131. The process of any one of claims 116, 117, 118, 123, 124, 125, 126, or 127, wherein the chimeric G protein comprises an invertebrate Gαq G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate Gαz protein beginning with the C-terminal amino acid of such vertebrate Gαz protein, wherein such number equals the number of amino acids deleted.

132. The process of any one of claims 116, 117, 118, 123, 124, 125, 126, or 127, wherein the chimeric

G protein comprises an invertebrate Gαq G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate Gαs protein beginning with the C-terminal amino acid of such vertebrate Gαs protein, wherein such number equals the number of amino acids deleted.

133. The process of any one of claims 116, 117, 118, 123, 124, 125, 126, or 127, wherein the chimeric G protein comprises an invertebrate Gαq G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate Gi3 protein beginning with the C-terminal amino acid of such vertebrate Gi3 protein, wherein such number equals the number of amino acids deleted.

134. The process of any one of claims 116, 117, 118, 123, 124, 125, 126, or 127, wherein the chimeric G protein comprises an *Caenorhabditis elegans* Gαq G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate G protein beginning with the C-terminal amino acid of such vertebrate G protein, wherein such number equals the number of amino acids deleted.

135. The process of any one of claims 116, 117, 118, 123, 124, 125, 126, or 127, wherein the chimeric G protein comprises a *Drosophila melanogaster* Gαq G protein, a *Limulus polyphemus* Gαq G protein, a *Patinopecten yessoensis* Gαq G protein, a *Loligo forbesi* Gαq G protein, a *Homarus americanus* Gαq G protein, a *Lymnaea stagnalis* Gαq G protein, a *Geodia cydonium* Gαq G protein, or a *Dictyostelium discoideum* $G\alpha_4$ G protein, from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate G protein beginning with the C-terminal amino acid of such vertebrate G protein, wherein such number equals the number of amino acids deleted.

136. The process of any one of claims 116, 117, 118, 123, 124, 125, 126, or 127, wherein the chimeric G protein has an amino acid sequence substantially the same as the amino acid sequence shown in (a) Figure 2, *C. elegans* $G\alpha_{q/z5}$ (SEQ ID NO: 1); (b) Figure 2, *C. elegans* $G\alpha_{q/z9}$ (SEQ ID NO: 2); (c) Figure 2, *C. elegans* $G\alpha_{q/s9}$ (SEQ ID NO: 3); (d) Figure 2, *C. elegans* $G\alpha_{q/s21}$ (SEQ ID NO: 4); (e) Figure 2, *C. elegans* $G\alpha_{q/i3(5)}$ (SEQ ID NO: 5); or (f) Figure 2, *D. melaongaster* $G\alpha_{q/zs}$ (SEQ ID NO: 41).

137. The process of any one of claims 116, 117, 118, 123, 124, 125, 126, or 127, wherein the cell is an insect cell.

138. The process of any one of claims 116, 117, 118, 123, 124, 125, 126, or 127, wherein the cell is a mammalian cell.

139. The process of claim 138, wherein the cell is nonneuronal in origin.

140. The process of claim 139, wherein the nonneuronal cell is a COS-7 cell, 293 human embryonic kidney cell, a CHO cell, a NIH-3T3 cell, a mouse Y1 cell, or a LM(tk-) cell.

141. A process of screening a plurality of independent clones not known to include a clone encoding a mammalian G protein-coupled receptor, to identify and isolate a clone encoding a mammalian G protein-coupled receptor, which comprises:

(a) contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a plurality of independent clones with a ligand, under conditions permitting activation of a mammalian G protein-coupled receptor;

(b) determining whether the ligand activates the cells expressing the plurality of independent clones and the chimeric G protein; and if so

(c) isolating the single clone which expresses the mammalian G protein-coupled receptor activated by the ligand, so as to thereby identify any clone included in the

plurality of clones as encoding a mammalian G protein-coupled receptor.

142. A process of screening a plurality of independent clones not known to include a clone encoding a mammalian G protein-coupled receptor, to identify and isolate a clone encoding a mammalian G protein-coupled receptor, which comprises:

(a) contacting cells transfected with and expressing DNA encoding a chimeric G protein and expressing DNA encoding a plurality of independent clones with a ligand, under conditions permitting specific binding to a mammalian G protein-coupled receptor;

(b) determining whether the ligand specifically binds to the cells expressing the plurality of independent clones and the chimeric G protein; and if so

(c) isolating the single clone which expresses the mammalian G protein-coupled receptor which specifically binds to the ligand, so as to thereby identify any clone included in the plurality of clones as encoding a mammalian G protein-coupled receptor.

143. The process of claim 141 or 142, wherein the DNA encoding the plurality of independent clones is endogenous to the cell.

144. The process of claim 141 or 142, wherein the DNA encoding the plurality of independent clones is transfected into the cell.

145. The process of claim 141 or 142, wherein the chimeric G protein comprises an invertebrate Gαq G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate G protein beginning with the C-terminal amino acid of such vertebrate G protein, wherein such number equals the number of amino acids deleted.

146. The process of claim 141 or 142, wherein the chimeric G protein comprises an invertebrate Gαq G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate Gαz protein beginning with the C-terminal amino acid of such vertebrate Gαz protein, wherein such number equals the number of amino acids deleted.

147. The process of claim 141 or 142, wherein the chimeric G protein comprises an invertebrate Gαq G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate Gαs protein beginning with the C-terminal amino

acid of such vertebrate Gαs protein, wherein such number equals the number of amino acids deleted.

148. The process of claim 141 or 142, wherein the chimeric G protein comprises an invertebrate Gαq G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate Gαi3 protein beginning with the C-terminal amino acid of such vertebrate Gαi3 protein, wherein such number equals the number of amino acids deleted.

149. The process of claim 141 or 142, wherein the chimeric G protein comprises an *Caenorhabditis elegans* Gαq G protein from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate G protein beginning with the C-terminal amino acid of such vertebrate G protein, wherein such number equals the number of amino acids deleted.

150. The process of 141 or 142, wherein the chimeric G protein comprises a *Drosophila melanogaster* Gαq G protein, a *Limulus polyphemus* Gαq G protein, a *Patinopecten yessoensis* Gαq G protein, a *Loligo forbesi* Gαq G protein, a *Homarus americanus* Gαq G protein, a *Lymnaea stagnalis* Gαq G protein, a *Geodia cydonium* Gαq G protein, or a *Dictyostelium discoideum* $G\alpha_4$ G

protein, from which at least five, but not more than twenty-one, contiguous amino acids beginning with the C-terminal amino acid have been deleted and replaced by a number of contiguous amino acids present in a vertebrate G protein beginning with the C-terminal amino acid of such vertebrate G protein, wherein such number equals the number of amino acids deleted.

151. The process of claim 141 or 142, wherein the chimeric G protein has an amino acid sequence substantially the same as the amino acid sequence shown in (a) Figure 2, *C. elegans* $G\alpha_{q/z5}$ (SEQ ID NO: 1); (b) Figure 2, *C. elegans* $G\alpha_{q/z9}$ (SEQ ID NO: 2); (c) Figure 2, *C. elegans* $G\alpha_{q/s9}$ (SEQ ID NO: 3); (d) Figure 2, *C. elegans* $G\alpha_{q/s21}$ (SEQ ID NO: 4); (e) Figure 2, *C. elegans* $G\alpha_{q/i3(5)}$ (SEQ ID NO: 5); or (f) Figure 2, *D. melaongaster* $G\alpha_{q/zs}$ (SEQ ID NO: 41).

152. The process of claim 141 or 142, wherein the cell is an insect cell.

153. The process of claim 141 or 142, wherein the cell is a mammalian cell.

154. The process of claim 153, wherein the cell is nonneuronal in origin.

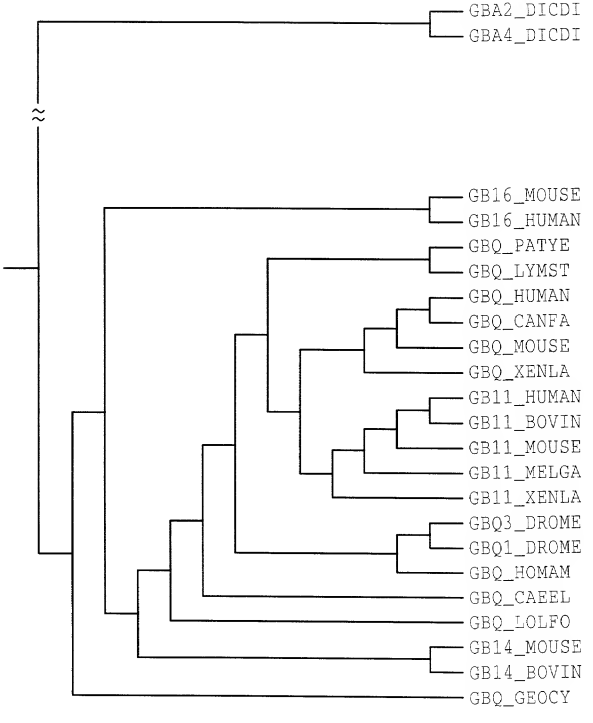
155. The process of claim 154, wherein the nonneuronal cell is a COS-7 cell, 293 human embryonic kidney cell, a CHO cell, a NIH-3T3 cell, a mouse Y1 cell, or a LM(tk-) cell.

CHIMERIC G PROTEINS AND USES THEREOF

Abstract Of The Disclosure

This invention provides isolated nucleic acids encoding chimeric G proteins, vectors comprising nucleic acids encoding chimeric G proteins, cells comprising such vectors, processes of determining agonists and antagonists of mammalian G protein-coupled receptors utilizing chimeric G proteins, processes of determining compounds which bind to mammalian G protein-coupled receptors utilizing chimeric G proteins, processes for making a composition of matter which specifically binds to a mammalian G protein-coupled receptor utilizing chimeric G proteins, processes for preparing a composition which comprises admixing a carrier and a pharmaceutically effective amount of a chemical compound identified by a process of the invention utilizing chimeric G proteins, processes of identifying a ligand for a mammalian G protein-coupled receptor utilizing chimeric G proteins, and processes of screening a plurality of independent clones to identify and isolate a clone encoding a mammalian G protein-coupled receptor utilizing chimeric G proteins.

FIGURE 1 1/8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GBQ_GEOCY

FIGURE 2A

C. elegans $G\alpha_{q/s5}$

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  1 MACCLSEEAR EQKRINQEIE KQLQRDKRNA RRELKLLLLG TGESGKSTFI KQMRIIHGQG
 61 YSEEDKRAHI RLVYQNVFMA IQSMIRAMDT LDIKFGNESE ELQEKAAVVR EVDFESVTSF
121 EEPYVSYIKE LWEDSGIQEC YDRRREYQLT DSAKYYLSDL RRLAVPDYLP TEQDILRVRV
181 PTTGIIEYPF DLEQIIFRMV DVGGQRSERR KWIHCFENVT SIMFLVALSE YDQVLVECDN
241 ENRMEESKAL FRTIITYPWF TNSSVILFLN KKDLLEEKIL YSHLADYFPE YDGPPRDPIA
301 AREFILKMFV DLNPDADKII YSHFTCATDT ENIRFVFAAV KDTILQHNLK YIGLC
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C. elegans $G\alpha_{q/s9}$

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 61 YSEEDKRAHI RLVYQNVFMA IQSMIRAMDT LDIKFGNESE ELQEKAAVVR EVDFESVTSF
121 EEPYVSYIKE LWEDSGIQEC YDRRREYQLT DSAKYYLSDL RRLAVPDYLP TEQDILRVRV
181 PTTGIIEYPF DLEQIIFRMV DVGGQRSERR KWIHCFENVT SIMFLVALSE YDQVLVECDN
241 ENRMEESKAL FRTIITYPWF TNSSVILFLN KKDLLEEKIL YSHLADYFPE YDGPPRDPIA
301 AREFILKMFV DLNPDADKII YSHFTCATDT ENIRFVFAAV KDTILQNNLK YIGLC
```

C. elegans $G\alpha_{q/s9}$

```
  1 MACCLSEEAR EQKRINQEIE KQLQRDKRNA RRELKLLLLG TGESGKSTFI KQMRIIHGQG
 61 YSEEDKRAHI RLVYQNVFMA IQSMIRAMDT LDIKFGNESE ELQEKAAVVR EVDFESVTSF
121 EEPYVSYIKE LWEDSGIQEC YDRRREYQLT DSAKYYLSDL RRLAVPDYLP TEQDILRVRV
181 PTTGIIEYPF DLEQIIFRMV DVGGQRSERR KWIHCFENVT SIMFLVALSE YDQVLVECDN
241 ENRMEESKAL FRTIITYPWF TNSSVILFLN KKDLLEEKIL YSHLADYFPE YDGPPRDPIA
301 AREFILKMFV DLNPDADKII YSHFTCATDT ENIRFVFAAV KDTILQMHLR QYELL
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C. elegans $G\alpha_{q/s21}$

```
  1 MACCLSEEAR EQKRINQEIE KQLQRDKRNA RRELKLLLLG TGESGKSTFI KQMRIIHGQG
 61 YSEEDKRAHI RLVYQNVFMA IQSMIRAMDT LDIKFGNESE ELQEKAAVVR EVDFESVTSF
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181 PTTGIIEYPF DLEQIIFRMV DVGGQRSERR KWIHCFENVT SIMFLVALSE YDQVLVECDN
241 ENRMEESKAL FRTIITYPWF TNSSVILFLN KKDLLEEKIL YSHLADYFPE YDGPPRDPIA
301 AREFILKMFV DLNPDADKII YSHFTCATDT ENIRRVFNDC RDIIQRMHLR QYELL
```

C. elegans $G\alpha_{q/s13(5)}$

```
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181 PTTGIIEYPF DLEQIIFRMV DVGGQRSERR KWIHCFENVT SIMFLVALSE YDQVLVECDN
241 ENRMEESKAL FRTIITYPWF TNSSVILFLN KKDLLEEKIL YSHLADYFPE YDGPPRDPIA
301 AREFILKMFV DLNPDADKII YSHFTCATDT ENIRFVFAAV KDTILQHNLK ECGLY
```

FIGURE 2B

D. melanogaster $G\alpha_{q/z5}$

```
  1 MECCLSEEAK EQKRINQEIE KQLRRDKRDA RRELKLLLLG TGESGKSTFI KQMRIIHGSG
 61 YSDEDKRGYI KLVFQNIFMA MQSMIKAMDM LKISYGQGEH SELADLVMSI DYETVTTFED
121 PYLNAIKTLW DDAGIQECYD RRREYQLTDS AKYYLKDLDR VAQPAYLPTE QDILRVRVPT
181 TGIIEYPFDL EEIRFRMVDV GGQRSERRKW IHCFENVTSI IFLVALSEYD QILFESDNEN
241 RMEESKALFR TIITYPWFQN SSVILFLNKK DLLEEKIMYS HLVDYFPEYD GPQRDAITAR
301 EFILRMFVDL NPDSEKIIYS HFTCATDTEN IRFVFAAVKD TILQSNLKYI GLC
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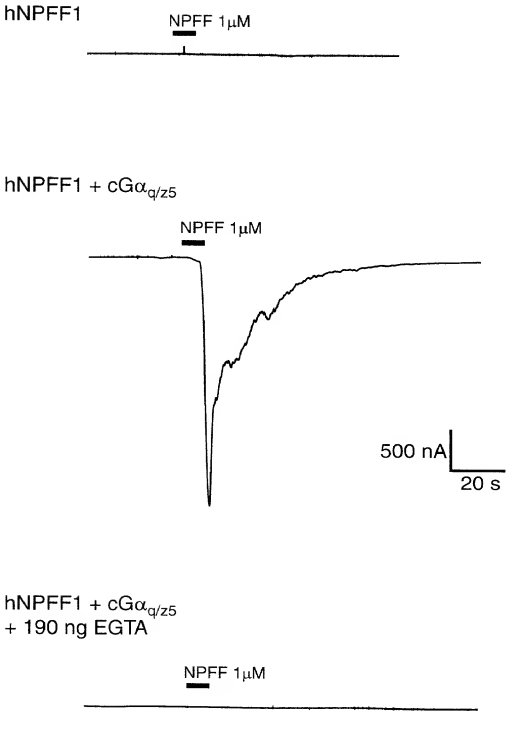
FIGURE 3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hNPFF1 + cGα$_{q/z5}$
NPFF 1μM
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hNPFF1 + cGα$_{q/z5}$
+ 190 ng EGTA
NPFF 1μM

FIGURE 4

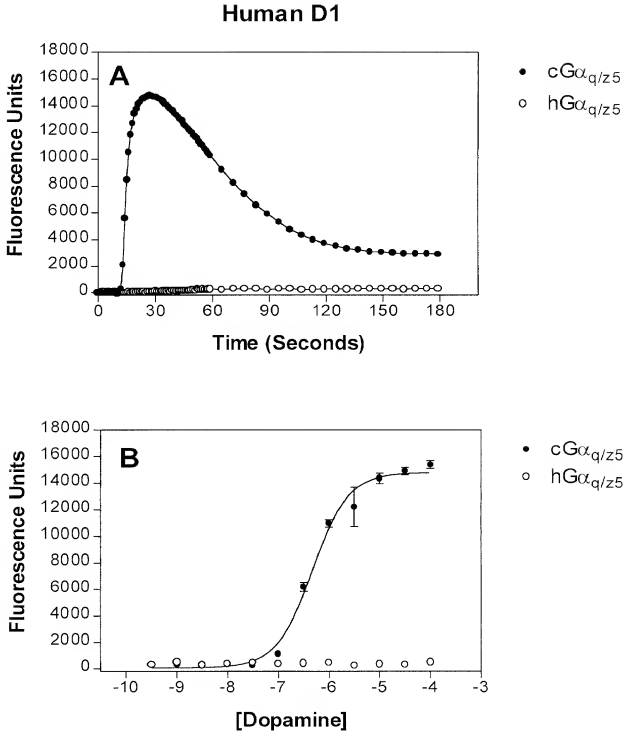
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FIGURE 5A

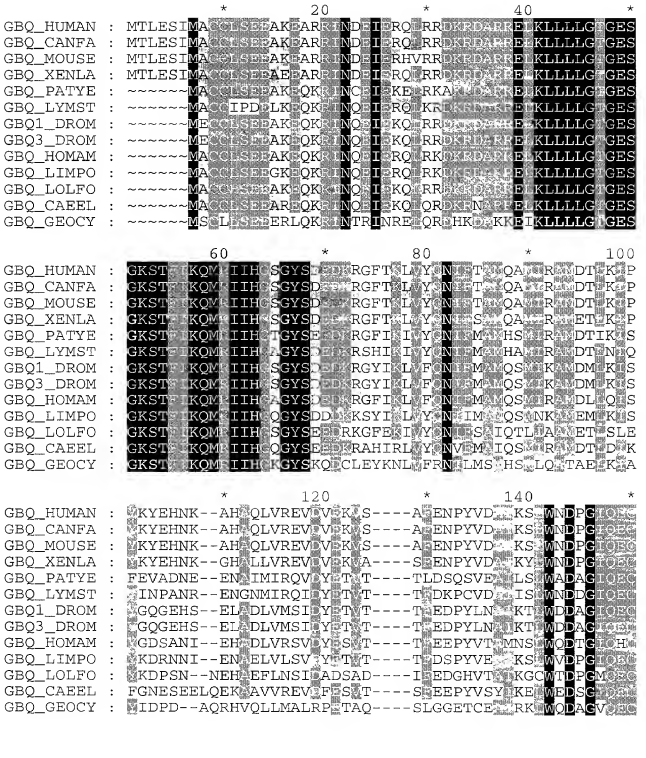
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FIGURE 5B

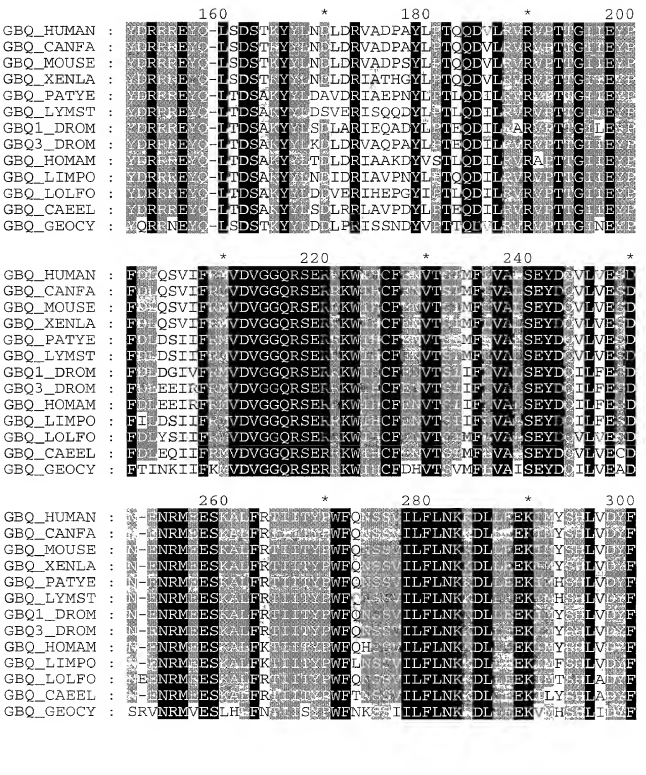
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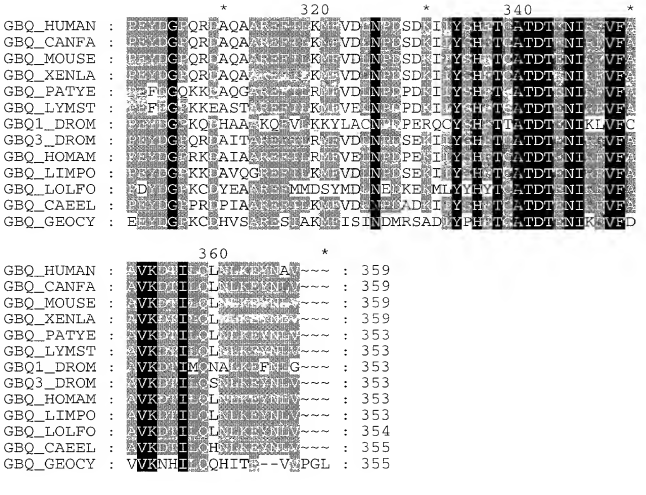
FIGURE 5C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GBQ_LOLFO
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GBQ_GEOCY

59896/JPW/ADM

DECLARATION AND POWER OF ATTORNEY

As a below-named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled.

CHIMERIC G PROTEINS AND USES THEREOF

the specification of which:
(check one)

X *is attached hereto*

___ *was filed on* _____ *as*

Application Serial No _____

and was amended December 23, 1999
(if applicable)

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above

I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119 (a)-(d) or Section 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT International Application which designated at least one country other than the United States, listed below I have also identified below any foreign application for patent or inventor's certificate, or PCT International Application having a filing date before that of the earliest application from which priority is claimed:

Prior Foreign Application(s)			*Priority Claimed*	
Number	*Country*	*Filing Date*	*Yes*	*No*
N/A				

I hereby claim the benefit under Title 35, United States Code, Section 119(e) of any United States provisional application(s) listed below:

Provisional Application No.	*Filing Date*	*Status*
N/A		

I hereby claim the benefit under Title 35, United States Code, Section 120 of any United States Application(s), or Section 365(c) of any PCT International Application(s) designating the United States listed below. Insofar as this application discloses and claims subject matter in addition to that disclosed in any such prior Application in the manner provided by the first paragraph of Title 35, United States Code, Section 112, I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56 which became available between the filing date(s) of such prior Application(s) and the national or PCT international filing date of this application:

Application Serial No.	*Filing Date*	*Status*
N/A		

And I hereby appoint

John P. White (Reg. No. 28,678); Christopher C. Dunham (Reg. No. 22,031); Norman H. Zivin (Reg. No. 25,385); Jay H. Maioli (Reg. No. 27,213); William E. Pelton (Reg. No. 25, 702); Robert D. Katz (Reg. No. 30,141); Peter J. Phillips (Reg. No. 29, 691) Wendy E. Miller (Reg. No. 35, 615); Richard S. Milner (Reg. No. 33,970); Robert T. Maldonado (Reg. No. 38,232); Paul Teng (Reg. No. 40, 837); Gary J. Gershik (Reg. No. 39,992); Richard F. Jaworski (Reg. No. 33,515); Elizabeth M. Wieckowski (Reg. No. 42,226); and Pedro C. Fernandez (Reg. No. 41,741)

and each of them, all c/o Cooper & Dunham LLP, 1185 Avenue of the Americas, New York, New York 10036, my attorneys, each with full power of substitution and revocation, to prosecute this application, to make alterations and amendments therein, to receive the patent, to transact all business in the Patent and Trademark Office connected therewith and to file any International Applications which are based thereon under the provisions of the Patent Cooperation Treaty.

Please address all communications, and direct all telephone call, regarding this application to

John P. White, Esq. *Reg. No.* 28,678
Cooper & Dunham LLP
1185 Avenue of the Americas
New York, New York 10036
Tel. (212) 278-0400

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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Inventor's signature Kenneth A. Jones

Citizenship U.S.A. *Date of signature* December 22, 1999

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Post Office Address same as residence address

Full name of sole or first joint inventor Mary W. Walker

Inventor's signature Mary W. Walker

Citizenship U.S.A. *Date of signature* December 20, 1999

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Post Office Address same as residence address

Full name of sole or first joint inventor Joseph Tamm

Inventor's signature Joseph Tamm

Citizenship U.S.A. *Date of signature* 12/22/99

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Post Office Address same as residence address

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Inventor's signature Theresa A. Branchek

Citizenship U.S.A. Date of signature 12/22/99

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Post Office Address same as residence address

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Inventor's signature

Citizenship France Date of signature 12/22/99

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Post Office Address same as residence address

Full name of joint inventor (if any)

Inventor's signature

Citizenship Date of signature

Residence

Post Office Address

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Walker, Mary W.
Tamm, Joseph
Branchek, Theresa A.
Gerald, Christophe P.G.

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Tyr Pro Trp Phe Thr Asn Ser Ser Val Ile Leu Phe Leu Asn Lys Lys
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Tyr Ser His Phe Thr Cys Ala Thr Asp Thr Glu Asn Ile Arg Phe Val
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Phe Ile Lys Gln Met Arg Ile Ile His Gly Gln Gly Tyr Ser Glu Glu
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         35                  40                  45

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Tyr Pro Trp Phe Thr Asn Ser Ser Val Ile Leu Phe Leu Asn Lys Lys
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275 280 285

Pro Glu Tyr Asp Gly Pro Pro Arg Asp Pro Ile Ala Ala Arg Glu Phe
290 295 300

Ile Leu Lys Met Phe Val Asp Leu Asn Pro Asp Ala Asp Lys Ile Ile
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Tyr Ser His Phe Thr Cys Ala Thr Asp Thr Glu Asn Ile Arg Phe Val
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Gln Glu Ile Glu Lys Gln Leu Gln Arg Asp Lys Arg Asn Ala Arg Arg
             20                  25                  30

Glu Leu Lys Leu Leu Leu Leu Gly Thr Gly Glu Ser Gly Lys Ser Thr
         35                  40                  45

Phe Ile Lys Gln Met Arg Ile Ile His Gly Gln Gly Tyr Ser Glu Glu
     50                  55                  60

Asp Lys Arg Ala His Ile Arg Leu Val Tyr Gln Asn Val Phe Met Ala
 65                  70                  75                  80

Ile Gln Ser Met Ile Arg Ala Met Asp Thr Leu Asp Ile Lys Phe Gly
                 85                  90                  95

Asn Glu Ser Glu Glu Leu Gln Glu Lys Ala Ala Val Val Arg Glu Val
            100                 105                 110

Asp Phe Glu Ser Val Thr Ser Phe Glu Glu Pro Tyr Val Ser Tyr Ile
        115                 120                 125

Lys Glu Leu Trp Glu Asp Ser Gly Ile Gln Glu Cys Tyr Asp Arg Arg
    130                 135                 140

Arg Glu Tyr Gln Leu Thr Asp Ser Ala Lys Tyr Tyr Leu Ser Asp Leu
145                 150                 155                 160

Arg Arg Leu Ala Val Pro Asp Tyr Leu Pro Thr Glu Gln Asp Ile Leu
                165                 170                 175

Arg Val Arg Val Pro Thr Thr Gly Ile Ile Glu Tyr Pro Phe Asp Leu
            180                 185                 190
```

```
Glu Gln Ile Ile Phe Arg Met Val Asp Val Gly Gly Gln Arg Ser Glu
        195                 200                 205

Arg Arg Lys Trp Ile His Cys Phe Glu Asn Val Thr Ser Ile Met Phe
    210                 215                 220

Leu Val Ala Leu Ser Glu Tyr Asp Gln Val Leu Val Glu Cys Asp Asn
225                 230                 235                 240

Glu Asn Arg Met Glu Glu Ser Lys Ala Leu Phe Arg Thr Ile Ile Thr
                245                 250                 255

Tyr Pro Trp Phe Thr Asn Ser Ser Val Ile Leu Phe Leu Asn Lys Lys
            260                 265                 270

Asp Leu Leu Glu Glu Lys Ile Leu Tyr Ser His Leu Ala Asp Tyr Phe
        275                 280                 285

Pro Glu Tyr Asp Gly Pro Pro Arg Asp Pro Ile Ala Ala Arg Glu Phe
    290                 295                 300

Ile Leu Lys Met Phe Val Asp Leu Asn Pro Asp Ala Asp Lys Ile Ile
305                 310                 315                 320

Tyr Ser His Phe Thr Cys Ala Thr Asp Thr Glu Asn Ile Arg Arg Val
                325                 330                 335

Phe Asn Asp Cys Arg Asp Ile Ile Gln Arg Met His Leu Arg Gln Tyr
            340                 345                 350

Glu Leu Leu
        355
```

<210> 5
<211> 355
<212> PRT
<213> C. elegans

```
<400> 5
Met Ala Cys Cys Leu Ser Glu Glu Ala Arg Glu Gln Lys Arg Ile Asn
  1               5                  10                  15

Gln Glu Ile Glu Lys Gln Leu Gln Arg Asp Lys Arg Asn Ala Arg Arg
             20                  25                  30

Glu Leu Lys Leu Leu Leu Leu Gly Thr Gly Glu Ser Gly Lys Ser Thr
```

```
         35                  40                  45

Phe Ile Lys Gln Met Arg Ile Ile His Gly Gln Gly Tyr Ser Glu Glu
     50                  55                  60

Asp Lys Arg Ala His Ile Arg Leu Val Tyr Gln Asn Val Phe Met Ala
 65                  70                  75                  80

Ile Gln Ser Met Ile Arg Ala Met Asp Thr Leu Asp Ile Lys Phe Gly
                 85                  90                  95

Asn Glu Ser Glu Glu Leu Gln Glu Lys Ala Ala Val Val Arg Glu Val
            100                 105                 110

Asp Phe Glu Ser Val Thr Ser Phe Glu Glu Pro Tyr Val Ser Tyr Ile
        115                 120                 125

Lys Glu Leu Trp Glu Asp Ser Gly Ile Gln Glu Cys Tyr Asp Arg Arg
    130                 135                 140

Arg Glu Tyr Gln Leu Thr Asp Ser Ala Lys Tyr Tyr Leu Ser Asp Leu
145                 150                 155                 160

Arg Arg Leu Ala Val Pro Asp Tyr Leu Pro Thr Glu Gln Asp Ile Leu
                165                 170                 175

Arg Val Arg Val Pro Thr Thr Gly Ile Ile Glu Tyr Pro Phe Asp Leu
            180                 185                 190

Glu Gln Ile Ile Phe Arg Met Val Asp Val Gly Gly Gln Arg Ser Glu
        195                 200                 205

Arg Arg Lys Trp Ile His Cys Phe Glu Asn Val Thr Ser Ile Met Phe
    210                 215                 220

Leu Val Ala Leu Ser Glu Tyr Asp Gln Val Leu Val Glu Cys Asp Asn
225                 230                 235                 240

Glu Asn Arg Met Glu Glu Ser Lys Ala Leu Phe Arg Thr Ile Ile Thr
                245                 250                 255

Tyr Pro Trp Phe Thr Asn Ser Ser Val Ile Leu Phe Leu Asn Lys Lys
            260                 265                 270

Asp Leu Leu Glu Glu Lys Ile Leu Tyr Ser His Leu Ala Asp Tyr Phe
        275                 280                 285

Pro Glu Tyr Asp Gly Pro Pro Arg Asp Pro Ile Ala Ala Arg Glu Phe
```

```
    290                 295                 300

Ile Leu Lys Met Phe Val Asp Leu Asn Pro Asp Ala Asp Lys Ile Ile
305                 310                 315                 320

Tyr Ser His Phe Thr Cys Ala Thr Asp Thr Glu Asn Ile Arg Phe Val
                325                 330                 335

Phe Ala Ala Val Lys Asp Thr Ile Leu Gln His Asn Leu Lys Glu Cys
            340                 345                 350

Gly Leu Tyr
        355


<210> 6
<211> 359
<212> PRT
<213> Homo sapiens

<400> 6
Met Thr Leu Glu Ser Ile Met Ala Cys Cys Leu Ser Glu Glu Ala Lys
  1               5                  10                  15

Glu Ala Arg Arg Ile Asn Asp Glu Ile Glu Arg Gln Leu Arg Arg Asp
            20                  25                  30

Lys Arg Asp Ala Arg Arg Glu Leu Lys Leu Leu Leu Leu Gly Thr Gly
        35                  40                  45

Glu Ser Gly Lys Ser Thr Phe Ile Lys Gln Met Arg Ile Ile His Gly
    50                  55                  60

Ser Gly Tyr Ser Asp Glu Asp Lys Arg Gly Phe Thr Lys Leu Val Tyr
65                  70                  75                  80

Gln Asn Ile Phe Thr Ala Met Gln Ala Met Ile Arg Ala Met Asp Thr
                85                  90                  95

Leu Lys Ile Pro Tyr Lys Tyr Glu His Asn Lys Ala His Ala Gln Leu
            100                 105                 110

Val Arg Glu Val Asp Val Glu Lys Val Ser Ala Phe Glu Asn Pro Tyr
        115                 120                 125

Val Asp Ala Ile Lys Ser Leu Trp Asn Asp Pro Gly Ile Gln Glu Cys
    130                 135                 140
```

```
Tyr Asp Arg Arg Arg Glu Tyr Gln Leu Ser Asp Ser Thr Lys Tyr Tyr
145                 150                 155                 160

Leu Asn Asp Leu Asp Arg Val Ala Asp Pro Ala Tyr Leu Pro Thr Gln
                165                 170                 175

Gln Asp Val Leu Arg Val Arg Val Pro Thr Thr Gly Ile Ile Glu Tyr
            180                 185                 190

Pro Phe Asp Leu Gln Ser Val Ile Phe Arg Met Val Asp Val Gly Gly
        195                 200                 205

Gln Arg Ser Glu Arg Arg Lys Trp Ile His Cys Phe Glu Asn Val Thr
    210                 215                 220

Ser Ile Met Phe Leu Val Ala Leu Ser Glu Tyr Asp Gln Val Leu Val
225                 230                 235                 240

Glu Ser Asp Asn Glu Asn Arg Met Glu Glu Ser Lys Ala Leu Phe Arg
                245                 250                 255

Thr Ile Ile Thr Tyr Pro Trp Phe Gln Asn Ser Ser Val Ile Leu Phe
            260                 265                 270

Leu Asn Lys Lys Asp Leu Leu Glu Glu Lys Ile Met Tyr Ser His Leu
        275                 280                 285

Val Asp Tyr Phe Pro Glu Tyr Asp Gly Pro Gln Arg Asp Ala Gln Ala
    290                 295                 300

Ala Arg Glu Phe Ile Leu Lys Met Phe Val Asp Leu Asn Pro Asp Ser
305                 310                 315                 320

Asp Lys Ile Ile Tyr Ser His Phe Thr Cys Ala Thr Asp Thr Glu Asn
                325                 330                 335

Ile Arg Phe Val Phe Ala Ala Val Lys Asp Thr Ile Leu Gln Leu Asn
            340                 345                 350

Leu Lys Glu Tyr Asn Ala Val
        355
```

<210> 7
<211> 359
<212> PRT
<213> Canis familiaris

<400> 7

```
Met Thr Leu Glu Ser Ile Met Ala Cys Cys Leu Ser Glu Glu Ala Lys
  1               5                  10                  15

Glu Ala Arg Arg Ile Asn Asp Glu Ile Glu Arg Gln Leu Arg Arg Asp
            20                  25                  30

Lys Arg Asp Ala Arg Arg Glu Leu Lys Leu Leu Leu Leu Gly Thr Gly
        35                  40                  45

Glu Ser Gly Lys Ser Thr Phe Ile Lys Gln Met Arg Ile Ile His Gly
    50                  55                  60

Ser Gly Tyr Ser Asp Glu Asp Lys Arg Gly Phe Thr Lys Leu Val Tyr
65                  70                  75                  80

Gln Asn Ile Phe Thr Ala Met Gln Ala Met Ile Arg Ala Met Asp Thr
                85                  90                  95

Leu Lys Ile Pro Tyr Lys Tyr Glu His Asn Lys Ala His Ala Gln Leu
            100                 105                 110

Val Arg Glu Val Asp Val Glu Lys Val Ser Ala Phe Glu Asn Pro Tyr
        115                 120                 125

Val Asp Ala Ile Lys Ser Leu Trp Asn Asp Pro Gly Ile Gln Glu Cys
    130                 135                 140

Tyr Asp Arg Arg Arg Glu Tyr Gln Leu Ser Asp Ser Thr Lys Tyr Tyr
145                 150                 155                 160

Leu Asn Asp Leu Asp Arg Val Ala Asp Pro Ala Tyr Leu Pro Thr Gln
                165                 170                 175

Gln Asp Val Leu Arg Val Arg Val Pro Thr Thr Gly Ile Ile Glu Tyr
            180                 185                 190

Pro Phe Asp Leu Gln Ser Val Ile Phe Arg Met Val Asp Val Gly Gly
        195                 200                 205

Gln Arg Ser Glu Arg Arg Lys Trp Ile His Cys Phe Glu Asn Val Thr
    210                 215                 220

Ser Ile Met Phe Leu Val Ala Leu Ser Glu Tyr Asp Gln Val Leu Val
225                 230                 235                 240

Glu Ser Asp Asn Glu Asn Arg Met Glu Glu Ser Lys Ala Leu Phe Arg
                245                 250                 255
```

```
Thr Ile Ile Thr Tyr Pro Trp Phe Gln Asn Ser Ser Val Ile Leu Phe
            260                 265                 270

Leu Asn Lys Lys Asp Leu Leu Glu Glu Lys Ile Met Tyr Ser His Leu
        275                 280                 285

Val Asp Tyr Phe Pro Glu Tyr Asp Gly Pro Gln Arg Asp Ala Gln Ala
    290                 295                 300

Ala Arg Glu Phe Ile Leu Lys Met Phe Val Asp Leu Asn Pro Asp Ser
305                 310                 315                 320

Asp Lys Ile Ile Tyr Ser His Phe Thr Cys Ala Thr Asp Thr Glu Asn
                325                 330                 335

Ile Arg Phe Val Phe Ala Ala Val Lys Asp Thr Ile Leu Gln Leu Asn
            340                 345                 350

Leu Lys Glu Tyr Asn Leu Val
        355


<210> 8
<211> 359
<212> PRT
<213> Mus musculus

<400> 8
Met Thr Leu Glu Ser Ile Met Ala Cys Cys Leu Ser Glu Glu Ala Lys
  1               5                  10                  15

Glu Ala Arg Arg Ile Asn Asp Glu Ile Glu Arg His Val Arg Arg Asp
             20                  25                  30

Lys Arg Asp Ala Arg Arg Glu Leu Lys Leu Leu Leu Leu Gly Thr Gly
         35                  40                  45

Glu Ser Gly Lys Ser Thr Phe Ile Lys Gln Met Arg Ile Ile His Gly
     50                  55                  60

Ser Gly Tyr Ser Asp Glu Asp Lys Arg Gly Phe Thr Lys Leu Val Tyr
 65                  70                  75                  80

Gln Asn Ile Phe Thr Ala Met Gln Ala Met Ile Arg Ala Met Asp Thr
                85                  90                  95

Leu Lys Ile Pro Tyr Lys Tyr Glu His Asn Lys Ala His Ala Gln Leu
```

```
            100                 105                 110

Val Arg Glu Val Asp Val Glu Lys Val Ser Ala Phe Glu Asn Pro Tyr
        115                 120                 125

Val Asp Ala Ile Lys Ser Leu Trp Asn Asp Pro Gly Ile Gln Glu Cys
    130                 135                 140

Tyr Asp Arg Arg Arg Glu Tyr Gln Leu Ser Asp Ser Thr Lys Tyr Tyr
145                 150                 155                 160

Leu Asn Asp Leu Asp Arg Val Ala Asp Pro Ser Tyr Leu Pro Thr Gln
                165                 170                 175

Gln Asp Val Leu Arg Val Arg Val Pro Thr Thr Gly Ile Ile Glu Tyr
            180                 185                 190

Pro Phe Asp Leu Gln Ser Val Ile Phe Arg Met Val Asp Val Gly Gly
        195                 200                 205

Gln Arg Ser Glu Arg Arg Lys Trp Ile His Cys Phe Glu Asn Val Thr
    210                 215                 220

Ser Ile Met Phe Leu Val Ala Leu Ser Glu Tyr Asp Gln Val Leu Val
225                 230                 235                 240

Glu Ser Asp Asn Glu Asn Arg Met Glu Glu Ser Lys Ala Leu Phe Arg
                245                 250                 255

Thr Ile Ile Thr Tyr Pro Trp Phe Gln Asn Ser Ser Val Ile Leu Phe
            260                 265                 270

Leu Asn Lys Lys Asp Leu Leu Glu Glu Lys Ile Met Tyr Ser His Leu
        275                 280                 285

Val Asp Tyr Phe Pro Glu Tyr Asp Gly Pro Gln Arg Asp Ala Gln Ala
    290                 295                 300

Ala Arg Glu Phe Ile Leu Lys Met Phe Val Asp Leu Asn Pro Asp Ser
305                 310                 315                 320

Asp Lys Ile Ile Tyr Ser His Phe Thr Cys Ala Thr Asp Thr Glu Asn
                325                 330                 335

Ile Arg Phe Val Phe Ala Ala Val Lys Asp Thr Ile Leu Gln Leu Asn
            340                 345                 350

Leu Lys Glu Tyr Asn Leu Val
```

355

<210> 9
<211> 359
<212> PRT
<213> Xenopus laevis

<400> 9
Met Thr Leu Glu Ser Ile Met Ala Cys Cys Leu Ser Glu Glu Ala Glu
1 5 10 15

Glu Ala Arg Arg Ile Asn Asp Glu Ile Glu Arg Gln Leu Arg Arg Asp
20 25 30

Lys Arg Asp Ala Arg Arg Glu Leu Lys Leu Leu Leu Leu Gly Thr Gly
35 40 45

Glu Ser Gly Lys Ser Thr Phe Ile Lys Gln Met Arg Ile Ile His Gly
50 55 60

Ser Gly Tyr Ser Asp Glu Asp Lys Arg Gly Phe Thr Lys Leu Val Tyr
65 70 75 80

Gln Asn Ile Phe Ser Ala Met Gln Ala Met Ile Arg Ala Met Glu Thr
85 90 95

Leu Lys Ile Pro Tyr Lys Tyr Glu His Asn Lys Gly His Ala Leu Leu
100 105 110

Val Arg Glu Val Asp Val Glu Lys Val Ala Ser Phe Glu Asn Pro Tyr
115 120 125

Val Asp Ala Ile Lys Tyr Leu Trp Asn Asp Pro Gly Ile Gln Glu Cys
130 135 140

Tyr Asp Arg Arg Arg Glu Tyr Gln Leu Ser Asp Ser Thr Lys Tyr Tyr
145 150 155 160

Leu Asn Asp Leu Asp Arg Ile Ala Thr His Gly Tyr Leu Pro Thr Gln
165 170 175

Gln Asp Val Leu Arg Val Arg Val Pro Thr Thr Gly Ile Ile Glu Tyr
180 185 190

Pro Phe Asp Leu Gln Ser Val Ile Phe Arg Met Val Asp Val Gly Gly
195 200 205

```
Gln Arg Ser Glu Arg Arg Lys Trp Ile His Cys Phe Glu Asn Val Thr
    210                 215                 220

Ser Ile Met Phe Leu Val Ala Leu Ser Glu Tyr Asp Gln Val Leu Val
225                 230                 235                 240

Glu Ser Asp Asn Glu Asn Arg Met Glu Glu Ser Lys Ala Leu Phe Arg
                245                 250                 255

Thr Ile Ile Thr Tyr Pro Trp Phe Gln Asn Ser Ser Val Ile Leu Phe
            260                 265                 270

Leu Asn Lys Lys Asp Leu Leu Glu Glu Lys Ile Met Tyr Ser His Leu
        275                 280                 285

Val Asp Tyr Phe Pro Glu Tyr Asp Gly Pro Gln Arg Asp Ala Gln Ala
    290                 295                 300

Ala Arg Glu Phe Ile Leu Lys Met Phe Val Asp Leu Asn Pro Asp Ser
305                 310                 315                 320

Asp Lys Ile Ile Tyr Ser His Phe Thr Cys Ala Thr Asp Thr Glu Asn
                325                 330                 335

Ile Arg Phe Val Phe Ala Ala Val Lys Asp Thr Ile Leu Gln Leu Asn
            340                 345                 350

Leu Lys Glu Tyr Asn Leu Val
        355


<210> 10
<211> 353
<212> PRT
<213> Patinopecten yessoensis

<400> 10
Met Ala Cys Cys Leu Ser Glu Glu Ala Lys Glu Gln Lys Arg Ile Asn
  1               5                  10                  15

Cys Glu Ile Glu Lys Glu Leu Arg Lys Ala Lys Arg Asp Ala Arg Arg
             20                  25                  30

Glu Leu Lys Leu Leu Leu Leu Gly Thr Gly Glu Ser Gly Lys Ser Thr
         35                  40                  45

Phe Ile Lys Gln Met Arg Ile Ile His Gly Thr Gly Tyr Ser Glu Glu
     50                  55                  60
```

```
Asp Lys Arg Gly Phe Ile Lys Ile Val Tyr Gln Asn Ile Phe Met Ala
 65                  70                  75                  80

Met His Ser Met Ile Arg Ala Met Asp Thr Ile Lys Ile Ser Phe Glu
                85                  90                  95

Val Ala Asp Asn Glu Glu Asn Ala Ile Met Ile Arg Gln Val Asp Tyr
            100                 105                 110

Glu Thr Val Thr Thr Leu Asp Ser Gln Ser Val Glu Ala Ile Leu Ser
        115                 120                 125

Leu Trp Ala Asp Ala Gly Ile Gln Glu Cys Tyr Asp Arg Arg Arg Glu
    130                 135                 140

Tyr Gln Leu Thr Asp Ser Ala Lys Tyr Tyr Leu Asp Ala Val Asp Arg
145                 150                 155                 160

Ile Ala Glu Pro Asn Tyr Leu Pro Thr Leu Gln Asp Ile Leu Arg Val
                165                 170                 175

Arg Val Pro Thr Thr Gly Ile Ile Glu Tyr Pro Phe Asp Leu Asp Ser
            180                 185                 190

Ile Ile Phe Arg Met Val Asp Val Gly Gly Gln Arg Ser Glu Arg Arg
        195                 200                 205

Lys Trp Ile His Cys Phe Glu Asn Val Thr Ser Ile Met Phe Leu Val
    210                 215                 220

Ala Leu Ser Glu Tyr Asp Gln Val Leu Val Glu Ser Asp Asn Glu Asn
225                 230                 235                 240

Arg Met Glu Glu Ser Lys Ala Leu Phe Arg Thr Ile Ile Thr Tyr Pro
                245                 250                 255

Trp Phe Gln Asn Ser Ser Val Ile Leu Phe Leu Asn Lys Lys Asp Leu
            260                 265                 270

Leu Glu Glu Lys Ile Met His Ser His Leu Val Asp Tyr Phe Pro Glu
        275                 280                 285

Phe Asp Gly Gln Lys Lys Asp Ala Gln Gly Ala Arg Glu Phe Ile Leu
    290                 295                 300

Arg Met Phe Val Asp Leu Asn Pro Asp Pro Asp Lys Ile Ile Tyr Ser
305                 310                 315                 320
```

```
His Phe Thr Cys Ala Thr Asp Thr Glu Asn Ile Arg Phe Val Phe Ala
                325                 330                 335

Ala Val Lys Asp Thr Ile Leu Gln Leu Asn Leu Lys Glu Tyr Asn Leu
            340                 345                 350

Val
```

<210> 11
<211> 353
<212> PRT
<213> Lymnaea stagnalis

<400> 11

```
Met Ala Cys Cys Ile Pro Asp Glu Leu Lys Glu Gln Lys Arg Ile Asn
  1               5                  10                  15

Gln Glu Ile Glu Arg Gln Leu Lys Arg Asp Lys Arg Asp Ala Arg Arg
             20                  25                  30

Glu Leu Lys Leu Leu Leu Leu Gly Thr Gly Glu Ser Gly Lys Ser Thr
         35                  40                  45

Phe Ile Lys Gln Met Arg Ile Ile His Gly Ala Gly Tyr Ser Asp Glu
     50                  55                  60

Asp Lys Arg Ser His Ile Lys Ile Val Tyr Gln Asn Ile Phe Met Ala
 65                  70                  75                  80

Met His Ala Met Ile Arg Ala Met Asp Thr Leu Asn Ile Gln Tyr Ile
                 85                  90                  95

Asn Pro Ala Asn Arg Glu Asn Gly Asn Met Ile Arg Gln Ile Asp Tyr
            100                 105                 110

Glu Thr Val Thr Thr Phe Asp Lys Pro Cys Val Asp Ala Ile Ile Ser
        115                 120                 125

Leu Trp Asn Asp Asp Gly Ile Gln Glu Cys Tyr Asp Arg Arg Arg Glu
    130                 135                 140

Tyr Gln Leu Thr Asp Ser Ala Lys Tyr Tyr Leu Asp Ser Val Glu Arg
145                 150                 155                 160

Ile Ser Gln Gln Asp Tyr Leu Pro Thr Leu Gln Asp Ile Leu Arg Val
```

```
                165                 170                 175

Arg Val Pro Thr Thr Gly Ile Ile Glu Tyr Pro Phe Asp Leu Asp Ser
            180                 185                 190

Ile Ile Phe Arg Met Val Asp Val Gly Gly Gln Arg Ser Glu Arg Arg
        195                 200                 205

Lys Trp Ile His Cys Phe Glu Asn Val Thr Ser Ile Met Phe Leu Val
    210                 215                 220

Ala Leu Ser Glu Tyr Asp Gln Val Leu Val Glu Ser Asp Asn Glu Asn
225                 230                 235                 240

Arg Met Glu Glu Ser Lys Ala Leu Phe Arg Thr Ile Ile Thr Tyr Pro
                245                 250                 255

Trp Phe Gln Asn Ser Ser Val Ile Leu Phe Leu Asn Lys Lys Asp Leu
            260                 265                 270

Leu Glu Glu Lys Ile Met His Ser His Leu Val Asp Tyr Phe Pro Glu
        275                 280                 285

Phe Asp Gly Pro Lys Lys Glu Ala Ser Thr Ala Arg Glu Phe Ile Leu
    290                 295                 300

Lys Met Phe Val Glu Leu Asn Pro Asp Pro Asp Lys Ile Ile Tyr Ser
305                 310                 315                 320

His Phe Thr Cys Ala Thr Asp Thr Glu Asn Ile Arg Phe Val Phe Ala
                325                 330                 335

Ala Val Lys Asp Thr Ile Leu Gln Leu Asn Leu Lys Glu Tyr Asn Leu
            340                 345                 350

Val
```

<210> 12
<211> 353
<212> PRT
<213> Drosophila melanogaster

<400> 12

```
Met Glu Cys Cys Leu Ser Glu Glu Ala Lys Glu Gln Lys Arg Ile Asn
  1               5                  10                  15
```

```
Gln Glu Ile Glu Lys Gln Leu Arg Arg Asp Lys Arg Asp Ala Arg Arg
            20                  25                  30

Glu Leu Lys Leu Leu Leu Leu Gly Thr Gly Glu Ser Gly Lys Ser Thr
        35                  40                  45

Phe Ile Lys Gln Met Arg Ile Ile His Gly Ser Gly Tyr Ser Asp Glu
    50                  55                  60

Asp Lys Arg Gly Tyr Ile Lys Leu Val Phe Gln Asn Ile Phe Met Ala
65                  70                  75                  80

Met Gln Ser Met Ile Lys Ala Met Asp Met Leu Lys Ile Ser Tyr Gly
                85                  90                  95

Gln Gly Glu His Ser Glu Leu Ala Asp Leu Val Met Ser Ile Asp Tyr
            100                 105                 110

Glu Thr Val Thr Thr Phe Glu Asp Pro Tyr Leu Asn Ala Ile Lys Thr
        115                 120                 125

Leu Trp Asp Asp Ala Gly Ile Gln Glu Cys Tyr Asp Arg Arg Arg Glu
    130                 135                 140

Tyr Gln Leu Thr Asp Ser Ala Lys Tyr Tyr Leu Ser Asp Leu Ala Arg
145                 150                 155                 160

Ile Glu Gln Ala Asp Tyr Leu Pro Thr Glu Gln Asp Ile Leu Arg Ala
                165                 170                 175

Arg Val Pro Thr Thr Gly Ile Leu Glu Tyr Pro Phe Asp Leu Asp Gly
            180                 185                 190

Ile Val Phe Arg Met Val Asp Val Gly Gly Gln Arg Ser Glu Arg Arg
        195                 200                 205

Lys Trp Ile His Cys Phe Glu Asn Val Thr Ser Ile Ile Phe Leu Val
    210                 215                 220

Ala Leu Ser Glu Tyr Asp Gln Ile Leu Phe Glu Ser Asp Asn Glu Asn
225                 230                 235                 240

Arg Met Glu Glu Ser Lys Ala Leu Phe Arg Thr Ile Ile Thr Tyr Pro
                245                 250                 255

Trp Phe Gln Asn Ser Ser Val Ile Leu Phe Leu Asn Lys Lys Asp Leu
            260                 265                 270
```

```
Leu Glu Glu Lys Ile Met Tyr Ser His Leu Val Asp Tyr Phe Pro Glu
        275                 280                 285

Tyr Asp Gly Pro Lys Gln Asp His Ala Ala Ala Lys Gln Phe Val Leu
    290                 295                 300

Lys Lys Tyr Leu Ala Cys Asn Pro Asp Pro Glu Arg Gln Cys Tyr Ser
305                 310                 315                 320

His Phe Thr Thr Ala Thr Asp Thr Glu Asn Ile Lys Leu Val Phe Cys
                325                 330                 335

Ala Val Lys Asp Thr Ile Met Gln Asn Ala Leu Lys Glu Phe Asn Leu
            340                 345                 350

Gly
```

<210> 13
<211> 353
<212> PRT
<213> Drosophila melanogaster

```
<400> 13
Met Glu Cys Cys Leu Ser Glu Glu Ala Lys Glu Gln Lys Arg Ile Asn
  1               5                  10                  15

Gln Glu Ile Glu Lys Gln Leu Arg Arg Asp Lys Arg Asp Ala Arg Arg
             20                  25                  30

Glu Leu Lys Leu Leu Leu Leu Gly Thr Gly Glu Ser Gly Lys Ser Thr
         35                  40                  45

Phe Ile Lys Gln Met Arg Ile Ile His Gly Ser Gly Tyr Ser Asp Glu
     50                  55                  60

Asp Lys Arg Gly Tyr Ile Lys Leu Val Phe Gln Asn Ile Phe Met Ala
 65                  70                  75                  80

Met Gln Ser Met Ile Lys Ala Met Asp Met Leu Lys Ile Ser Tyr Gly
                85                  90                  95

Gln Gly Glu His Ser Glu Leu Ala Asp Leu Val Met Ser Ile Asp Tyr
            100                 105                 110

Glu Thr Val Thr Thr Phe Glu Asp Pro Tyr Leu Asn Ala Ile Lys Thr
        115                 120                 125
```

```
Leu Trp Asp Asp Ala Gly Ile Gln Glu Cys Tyr Asp Arg Arg Arg Glu
    130                 135                 140

Tyr Gln Leu Thr Asp Ser Ala Lys Tyr Tyr Leu Lys Asp Leu Asp Arg
145                 150                 155                 160

Val Ala Gln Pro Ala Tyr Leu Pro Thr Glu Gln Asp Ile Leu Arg Val
                165                 170                 175

Arg Val Pro Thr Thr Gly Ile Ile Glu Tyr Pro Phe Asp Leu Glu Glu
            180                 185                 190

Ile Arg Phe Arg Met Val Asp Val Gly Gly Gln Arg Ser Glu Arg Arg
        195                 200                 205

Lys Trp Ile His Cys Phe Glu Asn Val Thr Ser Ile Ile Phe Leu Val
    210                 215                 220

Ala Leu Ser Glu Tyr Asp Gln Ile Leu Phe Glu Ser Asp Asn Glu Asn
225                 230                 235                 240

Arg Met Glu Glu Ser Lys Ala Leu Phe Arg Thr Ile Ile Thr Tyr Pro
                245                 250                 255

Trp Phe Gln Asn Ser Ser Val Ile Leu Phe Leu Asn Lys Lys Asp Leu
            260                 265                 270

Leu Glu Glu Lys Ile Met Tyr Ser His Leu Val Asp Tyr Phe Pro Glu
        275                 280                 285

Tyr Asp Gly Pro Gln Arg Asp Ala Ile Thr Ala Arg Glu Phe Ile Leu
    290                 295                 300

Arg Met Phe Val Asp Leu Asn Pro Asp Ser Glu Lys Ile Ile Tyr Ser
305                 310                 315                 320

His Phe Thr Cys Ala Thr Asp Thr Glu Asn Ile Arg Phe Val Phe Ala
                325                 330                 335

Ala Val Lys Asp Thr Ile Leu Gln Ser Asn Leu Lys Glu Tyr Asn Leu
            340                 345                 350

Val
```

<210> 14

<211> 353
<212> PRT
<213> Homarus americanus

<400> 14

```
Met Ala Cys Cys Leu Ser Glu Glu Ala Lys Glu Gln Lys Arg Ile Asn
  1               5                  10                  15

Gln Glu Ile Glu Arg Gln Leu Arg Lys Asp Lys Arg Asp Ala Arg Arg
             20                  25                  30

Glu Leu Lys Leu Leu Leu Leu Gly Thr Gly Glu Ser Gly Lys Ser Thr
         35                  40                  45

Phe Ile Lys Gln Met Arg Ile Ile His Gly Ala Gly Tyr Ser Asp Glu
     50                  55                  60

Asp Lys Arg Gly Phe Ile Lys Leu Val Phe Gln Asn Ile Phe Met Ala
 65                  70                  75                  80

Met Gln Ser Met Ile Arg Ala Met Asp Leu Leu Gln Ile Ser Tyr Gly
                 85                  90                  95

Asp Ser Ala Asn Ile Glu His Ala Asp Leu Val Arg Ser Val Asp Tyr
            100                 105                 110

Glu Ser Val Thr Thr Phe Glu Glu Pro Tyr Val Thr Ala Met Asn Ser
        115                 120                 125

Leu Trp Gln Asp Thr Gly Ile Gln His Cys Tyr Asp Arg Arg Arg Glu
    130                 135                 140

Tyr Gln Leu Thr Asp Ser Ala Lys Tyr Tyr Leu Thr Asp Leu Asp Arg
145                 150                 155                 160

Ile Ala Ala Lys Asp Tyr Val Ser Thr Leu Gln Asp Ile Leu Arg Val
                165                 170                 175

Arg Ala Pro Thr Thr Gly Ile Ile Glu Tyr Pro Phe Asp Leu Glu Glu
            180                 185                 190

Ile Arg Phe Arg Met Val Asp Val Gly Gly Gln Arg Ser Glu Arg Arg
        195                 200                 205

Lys Trp Ile His Cys Phe Glu Asn Val Thr Ser Ile Ile Phe Leu Val
    210                 215                 220

Ala Leu Ser Glu Tyr Asp Gln Ile Leu Phe Glu Ser Asp Asn Glu Asn
```

```
225                 230                 235                 240

Arg Met Glu Glu Ser Lys Ala Leu Phe Lys Thr Ile Ile Thr Tyr Pro
                245                 250                 255

Trp Phe Gln His Ser Ser Val Ile Leu Phe Leu Asn Lys Lys Asp Leu
            260                 265                 270

Leu Glu Glu Lys Ile Met Tyr Ser His Leu Val Asp Tyr Phe Pro Glu
        275                 280                 285

Tyr Asp Gly Pro Arg Lys Asp Ala Ile Ala Ala Arg Glu Phe Ile Leu
    290                 295                 300

Arg Met Phe Val Glu Leu Asn Pro Asp Pro Glu Lys Ile Ile Tyr Ser
305                 310                 315                 320

His Phe Thr Cys Ala Thr Asp Thr Glu Asn Ile Arg Phe Val Phe Ala
                325                 330                 335

Ala Val Lys Asp Thr Ile Leu Gln Leu Asn Leu Lys Glu Tyr Asn Leu
            340                 345                 350

Val
```

<210> 15
<211> 353
<212> PRT
<213> Limulus polyphemus

<400> 15

```
Met Ala Cys Cys Leu Ser Glu Glu Gly Lys Glu Gln Lys Arg Ile Asn
  1               5                  10                  15

Gln Glu Ile Glu Arg Gln Leu Arg Lys Asp Lys Arg Asp Ala Arg Arg
             20                  25                  30

Glu Leu Lys Leu Leu Leu Leu Gly Thr Gly Glu Ser Gly Lys Ser Thr
         35                  40                  45

Phe Ile Lys Gln Met Arg Ile Ile His Gly Gln Gly Tyr Ser Asp Asp
     50                  55                  60

Asp Lys Lys Ser Tyr Ile Lys Leu Val Tyr Gln Asn Ile Ile Met Ala
 65                  70                  75                  80
```

```
Met Gln Ser Met Asn Lys Ala Met Glu Met Leu Lys Ile Ser Tyr Lys
                 85                  90                  95

Asp Arg Asn Asn Ile Glu Asn Ala Glu Leu Val Leu Ser Val Asp Tyr
            100                 105                 110

Glu Thr Val Thr Thr Phe Asp Ser Pro Tyr Val Glu Ala Ile Lys Ser
        115                 120                 125

Leu Trp Val Asp Pro Gly Ile Gln Glu Cys Tyr Asp Arg Arg Arg Glu
    130                 135                 140

Tyr Gln Leu Thr Asp Ser Ala Lys Tyr Tyr Leu Asn Asp Ile Asp Arg
145                 150                 155                 160

Ile Ala Val Pro Asn Tyr Leu Pro Thr Gln Gln Asp Ile Leu Arg Val
                165                 170                 175

Arg Val Pro Thr Thr Gly Ile Ile Glu Tyr Pro Phe Ile Leu Asp Ser
            180                 185                 190

Ile Ile Phe Arg Met Val Asp Val Gly Gly Gln Arg Ser Glu Arg Arg
        195                 200                 205

Lys Trp Ile His Cys Phe Glu Asn Val Thr Ser Ile Ile Phe Leu Val
    210                 215                 220

Ala Leu Ser Glu Tyr Asp Gln Ile Leu Phe Glu Ser Asp Asn Glu Asn
225                 230                 235                 240

Arg Met Glu Glu Ser Lys Ala Leu Phe Lys Thr Ile Ile Thr Tyr Pro
                245                 250                 255

Trp Phe Leu Asn Ser Ser Val Ile Leu Phe Leu Asn Lys Lys Asp Leu
            260                 265                 270

Leu Glu Glu Lys Ile Met Phe Ser His Leu Val Asp Tyr Phe Pro Glu
        275                 280                 285

Tyr Asp Gly Pro Lys Lys Asp Ala Val Gln Gly Arg Glu Phe Ile Leu
    290                 295                 300

Lys Met Phe Val Asp Leu Asn Pro Asp Ser Glu Lys Ile Ile Tyr Ser
305                 310                 315                 320

His Phe Thr Cys Ala Thr Asp Thr Glu Asn Ile Arg Phe Val Phe Ala
                325                 330                 335
```

```
Ala Val Lys Asp Thr Ile Leu Gln Leu Asn Leu Lys Glu Tyr Asn Leu
            340                 345                 350

Val
```

<210> 16
<211> 354
<212> PRT
<213> Loligo forbesi

<400> 16

```
Met Ala Cys Cys Leu Ser Glu Glu Ala Lys Glu Gln Lys Arg Ile Asn
  1               5                  10                  15

Gln Glu Ile Glu Lys Gln Leu Arg Arg Asp Lys Arg Asp Ala Arg Arg
             20                  25                  30

Glu Leu Lys Leu Leu Leu Leu Gly Thr Gly Glu Ser Gly Lys Ser Thr
         35                  40                  45

Phe Ile Lys Gln Met Arg Ile Ile His Gly Ser Gly Tyr Ser Glu Glu
     50                  55                  60

Asp Arg Lys Gly Phe Glu Lys Ile Val Tyr Gln Asn Ile Phe Ser Ala
 65                  70                  75                  80

Ile Gln Thr Leu Ile Ala Ala Met Glu Thr Leu Ser Leu Glu Tyr Lys
                 85                  90                  95

Asp Pro Ser Asn Asn Glu His Ala Glu Phe Leu Asn Ser Ile Asp Ala
            100                 105                 110

Asp Ser Ala Asp Ile Phe Glu Asp Gly His Val Thr Ala Ile Lys Gly
        115                 120                 125

Cys Trp Thr Asp Pro Gly Met Gln Glu Cys Tyr Asp Arg Arg Arg Glu
    130                 135                 140

Tyr Gln Leu Thr Asp Ser Ala Lys Tyr Tyr Leu Asp Asp Val Glu Arg
145                 150                 155                 160

Ile His Glu Pro Gly Tyr Ile Pro Thr Leu Gln Asp Ile Leu Arg Val
                165                 170                 175

Arg Val Pro Thr Thr Gly Ile Ile Glu Tyr Pro Phe Asp Leu Tyr Ser
            180                 185                 190
```

```
Ile Ile Phe Arg Met Val Asp Val Gly Gly Gln Arg Ser Glu Arg Arg
        195                 200                 205

Lys Trp Ile His Cys Phe Glu Asn Val Thr Ser Ile Met Phe Leu Val
    210                 215                 220

Ala Leu Ser Glu Tyr Asp Gln Val Leu Val Glu Ser Asp Asn Glu Glu
225                 230                 235                 240

Asn Arg Met Glu Glu Ser Lys Ala Leu Phe Arg Thr Ile Ile Thr Tyr
                245                 250                 255

Pro Trp Phe Gln Asn Ser Ser Val Ile Leu Phe Leu Asn Lys Lys Asp
            260                 265                 270

Leu Leu Glu Glu Lys Ile Met Thr Ser His Leu Ala Asp Tyr Phe Pro
        275                 280                 285

Asp Tyr Asp Gly Pro Lys Cys Asp Tyr Glu Ala Ala Arg Glu Phe Met
    290                 295                 300

Met Asp Ser Tyr Met Asp Leu Asn Glu Asp Lys Glu Lys Met Leu Tyr
305                 310                 315                 320

Tyr His Tyr Thr Cys Ala Thr Asp Thr Glu Asn Ile Arg Phe Val Phe
                325                 330                 335

Ala Ala Val Lys Asp Thr Ile Leu Gln Leu Asn Leu Lys Glu Tyr Asn
            340                 345                 350

Leu Val
```

<210> 17
<211> 355
<212> PRT
<213> Caenorhabditis elegans

```
<400> 17
Met Ala Cys Cys Leu Ser Glu Glu Ala Arg Glu Gln Lys Arg Ile Asn
  1               5                  10                  15

Gln Glu Ile Glu Lys Gln Leu Gln Arg Asp Lys Arg Asn Ala Arg Arg
             20                  25                  30

Glu Leu Lys Leu Leu Leu Leu Gly Thr Gly Glu Ser Gly Lys Ser Thr
```

```
         35                  40                  45

Phe Ile Lys Gln Met Arg Ile Ile His Gly Gln Gly Tyr Ser Glu Glu
     50                  55                  60

Asp Lys Arg Ala His Ile Arg Leu Val Tyr Gln Asn Val Phe Met Ala
 65                  70                  75                  80

Ile Gln Ser Met Ile Arg Ala Met Asp Thr Leu Asp Ile Lys Phe Gly
                 85                  90                  95

Asn Glu Ser Glu Glu Leu Gln Glu Lys Ala Ala Val Val Arg Glu Val
            100                 105                 110

Asp Phe Glu Ser Val Thr Ser Phe Glu Glu Pro Tyr Val Ser Tyr Ile
        115                 120                 125

Lys Glu Leu Trp Glu Asp Ser Gly Ile Gln Glu Cys Tyr Asp Arg Arg
    130                 135                 140

Arg Glu Tyr Gln Leu Thr Asp Ser Ala Lys Tyr Tyr Leu Ser Asp Leu
145                 150                 155                 160

Arg Arg Leu Ala Val Pro Asp Tyr Leu Pro Thr Glu Gln Asp Ile Leu
                165                 170                 175

Arg Val Arg Val Pro Thr Thr Gly Ile Ile Glu Tyr Pro Phe Asp Leu
            180                 185                 190

Glu Gln Ile Ile Phe Arg Met Val Asp Val Gly Gly Gln Arg Ser Glu
        195                 200                 205

Arg Arg Lys Trp Ile His Cys Phe Glu Asn Val Thr Ser Ile Met Phe
    210                 215                 220

Leu Val Ala Leu Ser Glu Tyr Asp Gln Val Leu Val Glu Cys Asp Asn
225                 230                 235                 240

Glu Asn Arg Met Glu Glu Ser Lys Ala Leu Phe Arg Thr Ile Ile Thr
                245                 250                 255

Tyr Pro Trp Phe Thr Asn Ser Ser Val Ile Leu Phe Leu Asn Lys Lys
            260                 265                 270

Asp Leu Leu Glu Glu Lys Ile Leu Tyr Ser His Leu Ala Asp Tyr Phe
        275                 280                 285

Pro Glu Tyr Asp Gly Pro Pro Arg Asp Pro Ile Ala Ala Arg Glu Phe
```

```
    290                 295                 300

Ile Leu Lys Met Phe Val Asp Leu Asn Pro Asp Ala Asp Lys Ile Ile
305                 310                 315                 320

Tyr Ser His Phe Thr Cys Ala Thr Asp Thr Glu Asn Ile Arg Phe Val
                325                 330                 335

Phe Ala Ala Val Lys Asp Thr Ile Leu Gln His Asn Leu Lys Glu Tyr
            340                 345                 350

Asn Leu Val
        355


<210> 18
<211> 355
<212> PRT
<213> Geodia cydonium

<400> 18
Met Ser Cys Leu Leu Ser Glu Glu Glu Arg Leu Gln Lys Arg Ile Asn
  1               5                  10                  15

Thr Arg Ile Asn Arg Glu Leu Gln Arg Asp His Lys Asp Ala Lys Lys
             20                  25                  30

Glu Ile Lys Leu Leu Leu Leu Gly Thr Gly Glu Ser Gly Lys Ser Thr
         35                  40                  45

Phe Ile Lys Gln Met Arg Ile Ile His Gly Lys Gly Tyr Ser Lys Gln
     50                  55                  60

Asp Cys Leu Glu Tyr Lys Asn Leu Val Phe Arg Asn Ile Leu Met Ser
 65                  70                  75                  80

Met His Ser Met Leu Gln Ala Thr Ala Glu Leu Lys Ile Ala Tyr Ile
                85                  90                  95

Asp Pro Asp Ala Gln Arg His Val Gln Leu Leu Met Ala Leu Arg Pro
            100                 105                 110

Glu Thr Ala Gln Ser Leu Gly Gly Glu Thr Cys Glu Ala Ile Arg Lys
        115                 120                 125

Leu Trp Gln Asp Ala Gly Val Gln Glu Cys Tyr Gln Arg Arg Asn Glu
    130                 135                 140
```

```
Tyr Gln Leu Ser Asp Ser Thr Lys Tyr Tyr Leu Asp Asp Leu Pro Arg
145                 150                 155                 160

Ile Ser Ser Asn Asp Tyr Val Pro Thr Thr Gln Asp Val Leu Arg Val
                165                 170                 175

Arg Val Pro Thr Thr Gly Ile Asn Glu Tyr Pro Phe Thr Ile Asn Lys
            180                 185                 190

Ile Ile Phe Lys Met Val Asp Val Gly Gly Gln Arg Ser Glu Arg Arg
        195                 200                 205

Lys Trp Ile His Cys Phe Asp His Val Thr Ser Val Met Phe Leu Val
    210                 215                 220

Ala Ile Ser Glu Tyr Asp Gln Ile Leu Val Glu Ala Asp Ser Arg Val
225                 230                 235                 240

Asn Arg Met Val Glu Ser Leu His Leu Phe Asn Thr Ile Ile Ser Tyr
                245                 250                 255

Pro Trp Phe Asn Lys Ser Ser Ile Ile Leu Phe Leu Asn Lys Lys Asp
            260                 265                 270

Leu Leu Glu Glu Lys Val Met His Ser His Leu Ile Asp Tyr Phe Glu
        275                 280                 285

Glu Tyr Asp Gly Pro Lys Cys Asp His Val Ser Ala Arg Glu Ser Ile
    290                 295                 300

Ala Lys Met Phe Ile Ser Ile Asn Asp Met Arg Ser Ala Asp Ile Tyr
305                 310                 315                 320

Pro His Phe Thr Cys Ala Thr Asp Thr Glu Asn Ile Lys Phe Val Phe
                325                 330                 335

Asp Val Val Lys Asn His Ile Leu Gln Gln His Ile Thr Glu Val Val
            340                 345                 350

Pro Gly Leu
        355
```

<210> 19
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer

<400> 19
gaatatgatg gaccccagag agatg 25

<210> 20
<211> 52
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer

<400> 20
gatcctcgag ttagcacagt ccgatgtact tcaggttcaa ctggaggatg gt 52

<210> 21
<211> 52
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer

<400> 21
gatcctcgag ttagtacagt ccgcatccct tcaggttcaa ctggaggatg gt 52

<210> 22
<211> 52
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer

<400> 22
gatcctcgag ttagtaaagc ccacattcct tcaggttcaa ctggaggatg gt 52

<210> 23
<211> 52
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer

<400> 23
gatcctcgag ttagagcagc tcgtattgct tcaggttcaa ctggaggatg gt 52

<210> 24
<211> 58
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer

<400> 24
ggaaaaaagc ggccgcttaa aacagtccgc agtccttcag gttcaactgg aggatggt 58

<210> 25
<211> 32
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer

<400> 25
ggggtaccgc cgccatggcc tgctgtttat cc 32

<210> 26
<211> 35
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer

<400> 26
gctctagatt acaccaagtt gtactccttc agatt 35

<210> 27
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer

<400> 27
ctctccgatc tccgacggct g 21

<210> 28
<211> 64
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer

<400> 28
ttctacagca taatctgaag tatatcggtt tgtgttaatc tagagggccc gtttaaaccc 60
gctg 64

<210> 29
<211> 64
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer

<400> 29
cagcgggttt aaacgggccc tctagattaa cacaaaccga tatacttcag attatgctgt 60
agaa 64

<210> 30
<211> 46
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer

<400> 30
cagcataatc tgaaggagtg tggattgtac taatctagag ggcccg 46

<210> 31
<211> 46
<212> DNA

<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer

<400> 31
cgggccctct agattagtac aatccacact ccttcagatt atgctg 46

<210> 32
<211> 69
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer

<400> 32
ggaaaaaagc ggccgcttag agcagctcgt attgcctcag gtgcatctgg aggatggtgt 60
ccttgacgg 69

<210> 33
<211> 63
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer

<400> 33
gctctagatt agagcagctc gtattgcctc aggtgcatct gtagaattgt gtctttgacg 60
gcg 63

<210> 34
<211> 63
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer

<400> 34
gctctagatt aacatagccc tatgtatttt agattattct gtagaattgt gtctttgacg 60
gcg 63

<210> 35
<211> 98
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer

<400> 35
gctctagatt agagcagctc gtattgcctc aggtgcatac gttgaataat gtcacgacag 60
tcattaaaaa cacgccgaat gttttccgta tcagtcgc 98

<210> 36
<211> 6
<212> PRT
<213> vertebrate

<400> 36
Met Thr Leu Glu Ser Ile
1 5

<210> 37
<211> 21
<212> PRT
<213> invertebrate

<400> 37
Phe Val Phe Ala Ala Val Lys Asp Thr Ile Leu Gln His Asn Leu Lys
1 5 10 15

Glu Tyr Asn Leu Val
20

<210> 38
<211> 21
<212> PRT
<213> vertebrate

<400> 38
Phe Val Phe Asp Ala Val Thr Asp Val Ile Ile Gln Asn Asn Leu Lys
1 5 10 15

Tyr Ile Gly Leu Cys
20

<210> 39
<211> 21
<212> PRT
<213> vertebrate

<400> 39
Arg Val Phe Asn Asp Cys Arg Asp Ile Ile Gln Arg Met His Leu Arg
1 5 10 15

Gln Tyr Glu Leu Leu
20

<210> 40
<211> 21
<212> PRT
<213> vertebrate

<400> 40
Phe Val Phe Asp Ala Val Thr Asp Val Ile Ile Lys Asn Asn Leu Lys
1 5 10 15

Glu Cys Gly Leu Tyr
20

<210> 41
<211> 353
<212> PRT
<213> Drosophila melanogaster

<400> 41
Met Glu Cys Cys Leu Ser Glu Glu Ala Lys Glu Gln Lys Arg Ile Asn
1 5 10 15

Gln Glu Ile Glu Lys Gln Leu Arg Arg Asp Lys Arg Asp Ala Arg Arg
20 25 30

Glu Leu Lys Leu Leu Leu Leu Gly Thr Gly Glu Ser Gly Lys Ser Thr
35 40 45

Phe Ile Lys Gln Met Arg Ile Ile His Gly Ser Gly Tyr Ser Asp Glu
50 55 60

Asp Lys Arg Gly Tyr Ile Lys Leu Val Phe Gln Asn Ile Phe Met Ala
65 70 75 80

```
Met Gln Ser Met Ile Lys Ala Met Asp Met Leu Lys Ile Ser Tyr Gly
                 85                  90                  95

Gln Gly Glu His Ser Glu Leu Ala Asp Leu Val Met Ser Ile Asp Tyr
            100                 105                 110

Glu Thr Val Thr Thr Phe Glu Asp Pro Tyr Leu Asn Ala Ile Lys Thr
        115                 120                 125

Leu Trp Asp Asp Ala Gly Ile Gln Glu Cys Tyr Asp Arg Arg Arg Glu
    130                 135                 140

Tyr Gln Leu Thr Asp Ser Ala Lys Tyr Tyr Leu Lys Asp Leu Asp Arg
145                 150                 155                 160

Val Ala Gln Pro Ala Tyr Leu Pro Thr Glu Gln Asp Ile Leu Arg Val
                165                 170                 175

Arg Val Pro Thr Thr Gly Ile Ile Glu Tyr Pro Phe Asp Leu Glu Glu
            180                 185                 190

Ile Arg Phe Arg Met Val Asp Val Gly Gly Gln Arg Ser Glu Arg Arg
        195                 200                 205

Lys Trp Ile His Cys Phe Glu Asn Val Thr Ser Ile Ile Phe Leu Val
    210                 215                 220

Ala Leu Ser Glu Tyr Asp Gln Ile Leu Phe Glu Ser Asp Asn Glu Asn
225                 230                 235                 240

Arg Met Glu Glu Ser Lys Ala Leu Phe Arg Thr Ile Ile Thr Tyr Pro
                245                 250                 255

Trp Phe Gln Asn Ser Ser Val Ile Leu Phe Leu Asn Lys Lys Asp Leu
            260                 265                 270

Leu Glu Glu Lys Ile Met Tyr Ser His Leu Val Asp Tyr Phe Pro Glu
        275                 280                 285

Tyr Asp Gly Pro Gln Arg Asp Ala Ile Thr Ala Arg Glu Phe Ile Leu
    290                 295                 300

Arg Met Phe Val Asp Leu Asn Pro Asp Ser Glu Lys Ile Ile Tyr Ser
305                 310                 315                 320

His Phe Thr Cys Ala Thr Asp Thr Glu Asn Ile Arg Phe Val Phe Ala
                325                 330                 335
```

Ala Val Lys Asp Thr Ile Leu Gln Ser Asn Leu Lys Tyr Ile Gly Leu
340 345 350

Cys

<210> 42
<211> 36
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer

<400> 42
cggggtaccc cggttagcat ggagtgctgt ttatcg 36

<210> 43
<211> 40
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer

<400> 43
ccggaattcc ggttagacca aattatattc cttaaggttc 40

<210> 44
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer

<400> 44
gagcatcgat tacgagaccg ttacc 25

<210> 45
<211> 53
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer

<400> 45
cggaattctt agcacagtcc gatgtactta aggttcgatt gcagaattgt gtc 53